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THE LIGNIN CONTENT OF SOME COMMON VEGETABLES, WITH OBSERVATIONS ON METHODS FOR THE DETERMINATION OF LIGNIN[1]

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In general, that fraction of foods and feeding-stuffs which is considered indigestible has been determined as crude fibre, a fraction which consists mainly of a mixture in variable proportions of cellulose, hemicellulose, and lignin. However, it has been shown by various investigators that the crude fibre fraction is, in fact, partly digestible, by ruminants at least, and that the relative digestibility of this fraction in different foodstuffs varies considerably (4). Furthermore, the crude fibre fraction does not include all the indigestible material in a given foodstuff since lignin, which according to present information is not appreciably digestible, is certainly removed in part by treatment with 1.25% sodium hydroxide solution. Recently the need for a more detailed analysis of the so-called indigestible fraction of foodstuffs has been emphasized by Crampton and Maynard (2), and by others. In view of its apparent indigestibility, and of its apparent effect as an encrusting material upon the availability of other components of foodstuffs, the accurate determination of the lignin content of food materials is of the first importance.

While considerable attention has been devoted to the determination of lignin in mature plant tissues, such as those of straw and hay, but little information is available concerning the lignin content of the more succulent tissues, such as those of vegetables. It was the purpose of this investigation to obtain information on the content and nature of the lignin fraction of some vegetables commonly used as foods by man.

The methods for the determination of lignin are at present highly empirical, and it has been well demonstrated that the nature and amount of the lignin fraction isolated depends to a considerable extent upon the pre-treatments employed in removing non-lignin interfering substances, as well as upon the method used in the actual isolation of the lignin residue. Hence it was thought desirable in the present study to investigate the results obtained by the use of a number of methods for the determination of lignin. The procedures employed were, (a) a modification of the 72% sulphuric acid method of Ost and Wilkening (7), (b) that proposed by Ross and Hill (13) as modified by Ross and Potter (14) and by Crampton

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and Maynard (2), and (c) the holocellulose method of van Beckum and Ritter (15), in which lignin is determined by difference. The products isolated by the first two of these procedures have been characterized by determination of their nitrogen and methoxyl contents.

MATERIALS AND METHODS

All samples were dried in an air oven at 105° C., the vegetable tissues being first cut into small pieces with a knife. After drying, unless otherwise stated, all samples were ground to pass a screen with one millimeter circular openings. Difficulty was experienced in grinding the dried samples of beets and rhubarb because of a tendency to stickiness. This was no doubt due to the high soluble sugar content and hygroscopic nature of these dried tissues.

Preliminary studies of the methods employed were carried out on a sample of red clover silage and one of oat straw.

The vegetables analyzed were rhubarb, asparagus, Swede turnips, spinach, green beans, carrots and beets. Details of the sampling and preparation for analysis are given below.

The rhubarb samples were gathered in the early morning and the most mature stalks were chosen on each date of sampling. The leaf blades and the below-ground portions of the petioles were discarded. For rhubarb sample A, taken on May 9, the average weight of the petioles was 44.3 grams, for sample B, gathered on June 3, it was 83.7 grams.

Asparagus samples consisted of stalks of as nearly as possible the same diameter and length. The below-ground portions of the stalks were discarded. For sample A, taken on May 16, the average length was 15 cm. and the mean diameter 0.9 cm. Sample B, gathered on June 20, averaged 20 cm. in length and 1.4 cm. in diameter. This latter sample was subdivided into three portions, B_1, the tips, consisting of the uppermost 8 cm. of the stalks, B_2, the middle sections, which were 6.5 cm. in length, and B_3, the butts, 5.5 cm. long.

Two samples of turnip tissues were analyzed. Sample A consisted of tissue affected with brown heart. This was obtained by slicing brown-hearted roots transversely and cutting out the affected portions with a knife. Sample B consisted of tissue taken from corresponding regions of roots showing no symptoms of brown-heart.

The spinach sample, which was gathered on June 24, represented all of the above-ground portions of the plants.

Green beans of the variety Kentucky Wonder were analyzed. The sample consisted of pods collected at their maximum maturity for edible purposes as green beans. In preparation for analysis small portions were discarded from the tip and stem ends of the pods.

Three samples of carrots, A, B and C, were studied. These were from plantings made on May 2, May 27, and July 6, respectively. Roots from all the plantings were harvested on the same date, about October 20. Roots of uniform size characteristic of the different plantings were selected for analysis.

Two samples of beets, A and B, consisting only of the main root portions of the plants, were investigated. Sample A was collected on July 23, at which time the average weight per root was 32.3 grams. Sample B was gathered on August 9, the average weight per root at that time being 100 grams. Beets of uniform size were chosen.

For determination of the lignin content by the 72% sulphuric acid method the dried tissues were first extracted for 30 hours with a 1 : 2 alcohol-benzene mixture. This treatment was followed by refluxing for 3 hours with water, 150 ml. of water per gram of dry tissue being used. The residue was finally refluxed for 3 hours with 1% HCl solution, using the same proportion of solution to tissue as for the aqueous extraction. It may be noted here that a limited comparison of pre-treatments with 1% hydrochloric and 5% sulphuric acid solutions indicated that differences in yield and nature of the lignin preparations isolated from different tissues were not consistent. The results obtained are given in Table 1. The lignin was isolated by treatment with 72% sulphuric acid for 2 hours at 10° C. In all the lignin determinations at this temperature digestion with the strong acid took place in an electric refrigerator where the temperature was somewhat variable although usually within the range 10° to 12° C. For those determinations mentioned later which were made at 20° C. the temperature was constant to less than ± 1° C. The 2-hour procedure at 10° C. is later shown to give somewhat higher yields of lignin than the 16-hour treatment at this temperature used for most of the analyses. Further comment concerning the differences between the products obtained by these two treatments is made later in this paper.

TABLE 1.—COMPARISON OF HYDROCHLORIC AND SULPHURIC ACID PRETREATMENTS IN THE DETERMINATION OF LIGNIN

Material	Treatment	Ash-free lignin as percentage of oven-dry tissue	Nitrogen in lignin	Methoxyl in lignin
		%	%	%
Clover silage	1% HCl	11.38 ± 0.24 (6)	3.40	6.77
	5% H_2SO_4	13.59 ± 0.36 (6)	3.77	6.51
Asparagus A	1% HCl	16.92 ± 0.25 (5)	8.27	0.77
	5% H_2SO_4	14.80 ± 0.35 (5)	8.49	1.02
Rhubarb B	1% HCl	20.44 ± 0.34 (5)	2.88	1.82
	5% H_2SO_4	21.50 ± 0.31 (4)	3.12	1.46

Tissue first extracted with alcohol-benzene and hot water.
Number of determinations given in parentheses following the average deviation.

In the early experiments with vegetable tissues it was found that, after being subjected to the series of pre-treatments outlined above, the residue dried to a hard mass which was difficult to sample for the lignin determination. This difficulty was overcome in the following manner: after filtering off the acid extract and washing free of acid with water, the residue was washed with alcohol solutions of increasing concentration, namely, 50%, followed by 75% and finally by 95% alcohol.

The treatment with 72% sulphuric acid was carried out as follows: one-half gram samples of the oven-dried extracted tissues were weighed into 50 ml. Erlenmeyer flasks fitted with corks through which glass rods were inserted. The samples and the 72% sulphuric acid were allowed to come to the temperature at which the treatment was to be given, when 15 ml. of the acid were added to each flask and the sample thoroughly mixed and wetted with the acid by stirring and tamping with the flattened ends of the stirring rods. The flasks were then stoppered to prevent dilution of the acid by the moisture of the air of the refrigerating chamber and allowed to stand, with occasional shaking, at the required temperature for the length of time being studied. The acid digestion was carried out for 2-hour and 16-hour periods at 10° C. and for 2 hours at 20° C. After standing with the 72% sulphuric acid for the required length of time the mixtures were diluted to 3% sulphuric acid content (by weight) and heated under reflux for 3 hours. The solutions were filtered while still hot, the lignin residue being collected either on Jena sintered-glass crucibles of porosity G-3, if nitrogen or methoxyl determinations were to be made, or on Gooch crucibles if ash was to be determined. When Gooch crucibles were used weighed amounts of Calite were added to facilitate filtration. The residues were washed with water and dried at 105° C.

The modification of the Ross-Hill method employed was that described by Crampton and Maynard (2) for the determination of lignin in feeds and faeces. A deviation from the procedure outlined by these authors was the use of a retentive filter paper (Whatman No. 50) instead of bolting silk for the filtration following the peptic digestion. The final separation of the lignin residues was by filtration on Gooch crucibles using Calite, or, when nitrogen or methoxyl determinations were to be made, using Jena sintered-glass crucibles. At no time during the separation of the lignin residues was the temperature allowed to exceed 70° C.

The holocellulose method of van Beckum and Ritter (15) was carried out as described by the authors.

The nitrogen content of the lignin preparations was determined by the micro-Kjeldahl method of Pregl (12), with the omission of the use of hydriodic acid and red phosphorus. The ammonia evolved was distilled into a solution of boric acid, and the latter was titrated back to the colour of a standard with 0.01 *N* HCl solution using methyl red as indicator.

The methoxyl content of the lignin preparations was determined by the Vieböck and Swappach modification of the Zeisel method as outlined by Clark (1).

EXPERIMENTAL RESULTS

In an attempt to decide upon the relative suitability of the three selected procedures for investigation of the lignin content of vegetable tissues, determinations of the apparent lignin content of three types of plant tissue were made by each. These tissues were of different degrees of maturity, succulence, and relative proportions of lignin to non-lignin components. The materials selected were: oat straw, as an example of highly lignified mature tissue; clover silage, as a material of an intermediate degree of lignification which had undergone fermentative alteration; and asparagus, a tissue considered to be relatively high in protein and low in

lignin content. The results obtained are given in Table 2. For the 72% sulphuric acid method the time of contact with the acid of this strength was 2 hours and the temperature 10° C. Results by the 72% sulphuric acid and the Ross-Hill methods are given as percentage of ash-free lignin in the oven-dry material.

TABLE 2.—APPARENT LIGNIN CONTENT BY DIFFERENT METHODS

Material	Percentage Ash-Free Lignin		Lignin by Difference
	72% sulphuric acid method	Ross-Hill method	Holocellulose method of van Beckum and Ritter
	%	%	
Oat straw			
Percentage lignin	10.90 ± 0.31 (8)	15.50 ± 0.78 (9)	12.76 ± 0.60 (2)
Percentage methoxyl in lignin	13.33	8.07	—
Percentage nitrogen in lignin	1.54	1.05	—
Clover silage			
Percentage lignin	11.38 ± 0.30 (6)	14.03 ± 1.48 (7)	15.28 (1)
Percentage methoxyl in lignin	6.77	5.11	—
Percentage nitrogen in lignin	3.40	3.83	—
Asparagus B_1			
Percentage lignin	17.08 ± 0.67 (3)	22.76 ± 2.29 (5)	20.80 (1)
Percentage methoxyl in lignin	0.58	0.95	—
Percentage nitrogen in lignin	10.72	10.32	—

Number of determinations given in parentheses following the average deviation.

It may be noted that the results obtained for oat straw using the 72% sulphuric acid method of isolation compare favourably with those recorded in the literature. Thus, Norman and Jenkins (6), using the 72% sulphuric acid method with a time of contact of 16 hours at less than 20° C. found the following values for the lignin content of oat straw as percentage of oven-dry tissue: 11.84, 12.04, 12.12 and 12.90%. Phillips *et al.* (10), using the 43% hydrochloric acid procedure, give the lignin content of oat straw as 10.27 and 10.87%. The values found by these latter workers represent lignin after correction for protein content (crude lignin − ash − $N \times 6.25$). Our value for the lignin content of oat straw by the 72% sulphuric acid method has been corrected for ash content, which was 4.8% of the crude lignin, but no attempt has been made to correct for the presence of nitrogenous impurities. The crude lignin residues for the clover silage and asparagus samples contained less than 1% ash.

When the results obtained by the three procedures are compared, it is seen that the 72% sulphuric acid method gives in all instances a lower and less variable yield of apparent lignin. Further, the Crampton and Maynard modification of the Ross-Hill procedure, in spite of the fact that it employs peptic digestion for the purpose of more efficient removal of protein, nevertheless yields a lignin residue of approximately the same percentage nitrogen content as does the 72% sulphuric acid method employing 1% HCl solution for this purpose. Indeed, in the residues from the clover silage and asparagus B_1 samples the absolute amounts of nitrogen are

higher for the Ross-Hill than for the 72% sulphuric acid method. The longer and more tedious peptic digestion procedure thus appears to have no advantage over dilute acid hydrolysis so far as preventing contamination of the lignin residue with nitrogenous materials is concerned. Further, both the higher yield of lignin residue obtained by the Ross-Hill procedure and its lower methoxyl content indicate greater contamination of this residue by non-lignin materials than is the case for the residue obtained by the 72% sulphuric acid procedure.

The holocellulose method of van Beckum and Ritter gave results somewhat higher than either of the other two methods under investigation. This probably may be attributed to partial removal of hemicellulose and protein along with the lignin. Nitrogen determinations on the materials analyzed before and after treatment by the holocellulose procedure showed that part, though not all, of the protein of the silage and the asparagus samples had been removed. Great difficulty was experienced in filtering and washing the holocellulose residue, which became more gelatinous as washing proceeded.

On the basis of the results recorded in Table 2 it was decided that, of the three procedures investigated, the 72% sulphuric acid method was preferable for the investigation of the lignin content of vegetable tissues.

The effects of time and temperature of contact with 72% sulphuric acid upon the yield and nature of the lignin residues were also investigated. The usual pre-treatments with alcohol-benzene, water, and 1% hydrochloric acid were given prior to attack by the strong acid. In two instances, rhubarb sample A, treatments 1a and 2a, the dried tissues were extracted with hot water for 3 hours and the residue again dried prior to the application of the regular pre-treatments. Contact with the 72% sulphuric acid for 2 hours at 10° C. constituted Treatment 1, 16 hours contact at 10° C. Treatment 2, and 2 hours at 20° C. Treatment 3. The results obtained are given in Table 3. The number of determinations made on each tissue is given in parentheses after the average deviation from the mean.

Comparing the results obtained by treatments 1 and 2, it is seen that the former gave markedly higher yields of lignin residue for 6 of the 10 tissues analyzed. The differences in yield for the 4 remaining tissues do not appear to be significant. This evidence of greater contamination of the lignin with the shorter time of contact with the strong acid is confirmed by the fact that, for 5 of the 7 tissues for which the methoxyl content of the lignin residues from both treatments were obtained, these values are lower for the residues resulting from the shorter period of contact. Increasing the time that the tissue remained in contact with the 72% sulphuric acid solution increased the percentage nitrogen content of the lignin residues for 6 of the 9 tissues for which this information was obtained. However, the absolute amounts of nitrogen in the residues remained essentially constant in 3 instances, rhubarb B, spinach, and beets A. Two hours contact with the acid at 20° gave higher yields of lignin residues than did 16 hours at 10° for 3 of the 6 tissues compared, the yields being essentially the same in the remaining 3 instances. The absolute nitrogen contents of the residues obtained under these conditions were essentially similar in 3 instances but appeared to be definitely higher for 2 tissues by treatment 3. However, Norman (5) has shown that the nitrogen content of lignin residues

TABLE 3.—EFFECT OF TIME AND TEMPERATURE OF CONTACT WITH 72 PER CENT SULPHURIC ACID

Material	Treatment	Ash-free lignin in oven-dry tissue	Methoxyl	Nitrogen
		%	%	%
Oat straw	1	10.90 ± 0.31 (8)	13.33	1.54
	2	11.33 ± 0.45 (3)	—	—
	3	11.72 ± 0.14 (4)	—	0.73
Turnip A	1	22.37 ± 0.22 (6)	0.35	2.41
	2	20.24 ± 0.47 (4)	0.70	4.29
Asparagus B_1	1	17.08 ± 0.67 (3)	0.85	9.15
	2	8.47 ± 0.49 (3)	1.88	7.61
Asparagus B_2	1	21.37 ± 0.46 (5)	0.58	10.72
	2	17.84 ± 0.49 (4)	1.64	8.75
	3	20.81 ± 0.26 (4)	—	7.66
Asparagus B_3	1	20.20 ± 0.39 (5)	1.10	5.78
	2	14.59 ± 0.25 (3)	1.66	4.00
Rhubarb A	1	9.48 ± 0.28 (5)	1.80	3.33
	1a	8.72 ± 0.42 (5)	1.32	3.76
	2a	9.57 ± 0.49 (4)	2.15	9.91
Rhubarb B	1	20.44 ± 0.32 (5)	1.82	2.88
	2	15.24 ± 0.30 (3)	1.85	4.11
Spinach	1	7.72 ± 0.66 (4)	1.20	6.35
	2	6.15 ± 1.05 (3)	1.06	7.13
	3	8.69 ± 0.66 (3)	—	7.85
String Beans	1	12.65 ± 0.61 (4)	—	6.54
	2	7.31 ± 0.54 (4)	0.84	8.48
	3	9.55 ± 0.30 (3)	—	8.56
Beets A	1	12.21 ± 0.09 (4)	—	4.97
	2	7.38 ± 0.11 (3)	—	8.00
	3	7.88 ± 0.12 (3)	1.68	6.62
Beets B	2	12.79 ± 0.10 (4)	0.80	5.22
	3	12.18 ± 0.25 (4)	—	5.07

is not an accurate index of the effect of nitrogenous substances upon the weight of apparent lignin obtained. Unfortunately, data were not obtained for the methoxyl contents of the residues from treatment 3. It is suggested that the high results by treatment 1 may be due, to a considerable degree, to incomplete dispersion of the hemicellulose-cellulose fraction with the shorter time of contact with the strong acid.

Consideration of the results recorded in Table 3 led to the conclusion that, of the three treatments investigated, the second, involving 16 hours contact with 72% sulphuric acid at 10° C., was to be preferred for the investigation of the lignin content of vegetable tissues. It may be noted that the variations in treatment employed had relatively little effect upon the yields of apparent lignin obtained from oat straw, the differences being comparatively small as compared with those found when these treatments were applied to more succulent tissues of relatively higher protein content.

The data for the yield and nature of the lignin residues obtained from the vegetables analyzed are accumulated in Table 4. All results are for tissues pre-treated with alcohol-benzene, water, and 1% hydrochloric acid, except for asparagus A, for which 5% sulphuric acid was used as the acid extractant in place of 1% hydrochloric. In all instances the lignin residue was obtained by treatment with 72% sulphuric acid. For samples marked (a) the time of contact was 2 hours at 10° C., for those marked (b) 2 hours at 20° C., and for all others it was 16 hours at 10°. As has been indicated in the discussion of the results of Table 3, there is reason to believe that the apparent lignin contents of samples marked (a) are relatively too high as compared with those of the other samples. All lignin values here reported have been corrected for ash content. This was less than 1% of the lignin residue in all instances except for that from the spinach sample, which contained 12.0% of ash.

Table 4.—The apparent lignin content of some vegetable tissues

Material	Ash-free lignin in oven-dry tissue	Methoxyl	Nitrogen	Remarks
	%	%	%	
Turnip A	20.24 ± 0.47 (4)	0.70	4.29	Affected with brown-heart.
Turnip B (a)	14.28 ± 0.14 (5)	0.32	1.64	Normal.
Rhubarb A	9.48 ± 0.28 (5)	1.80	3.33	Gathered May 9, 1940.
Rhubarb B	15.24 ± 0.30 (3)	1.85	4.11	Gathered June 3, 1940.
Asparagus A (a)	14.80 ± 0.35 (5)	1.02	8.49	Gathered May 16, 1940.
Asparagus B_1	8.47 ± 0.49 (3)	1.88	7.61	Gathered June 20, 1940: tip portions of stalks.
Asparagus B_2	17.84 ± 0.49 (4)	1.64	8.75	Gathered June 20, 1940: middle portions of stalks.
Asparagus B_3	14.59 ± 0.25 (3)	1.66	4.00	Gathered June 20, 1940: basal portions of stalks.
Spinach	6.15 ± 1.03 (3)	1.06	7.13	Gathered June 24, 1940.
String beans	7.31 ± 0.54 (4)	0.84	8.48	
Carrots C (a)	7.29 ± 0.10 (6)	0.65	2.44	Gathered about Oct. 20, 1939, sown July 6, 1939.
Carrots B (a)	6.75 ± 0.39 (8)	0.62	2.91	Gathered about Oct. 20, 1939, sown May 27, 1939.
Carrots A (a)	9.38 ± 0.27 (7)	0.51	1.78	Gathered about Oct. 20, 1939, sown May 2, 1939.
Beets A	7.38 ± 0.11 (3)	—	8.00	Gathered July 23, 1940.
Beets A (b)	7.88 ± 0.12 (3)	1.68	6.62	Gathered July 23, 1940.
Beets B	12.79 ± 0.10 (4)	0.80	5.22	Gathered August 9, 1940.
Beets B (b)	12.18 ± 0.25 (4)	—	5.07	Gathered August 9, 1940.

DISCUSSION

Certain observations may be made concerning the variability in apparent lignin content of the various samples of the same kind of plant tissue. Thus, turnip tissue affected with brown-heart is definitely higher in apparent lignin content than is normal tissue. This finding agrees with the cytological observations of Lachance (3) on normal and affected turnips. Again, rhubarb petioles and beet roots show a marked increase in apparent

lignin content with advancing maturity. This is particularly striking in the case of the beet root, for which the amount of apparent lignin as a percentage of the dried tissue nearly doubles within a period of about a fortnight. Contrasted with the preceding is the relative constancy of the apparent lignin content of the tissues of asparagus stalks and carrot roots of different stages of maturity. It is worthy of note that the middle section of the asparagus stalks contained a relatively higher apparent lignin content than the region below it which had more recently emerged from the soil. It may be noted that the lignin content of carrot roots found by the 72% sulphuric acid method here employed is considerably greater than that found for similar tissues by Platenius (11) using the 43% hydrochloric acid procedure of Phillips. Platenius records apparent lignin contents for carrot tissue of 2.98% (June 16) to 1.95% (October 15) on roots grown from seed sown on May 1. However, the figures given are for lignin content corrected for protein by subtracting nitrogen × 6.25, a procedure the accuracy of which may be questioned. Applying the same correction to our data for the lignin content of carrots the percentages obtained become: sample C, 6·20; sample B, 5.53; and sample A, 8.35, values still very much higher than those obtained by Platenius. However, our lignin values for carrots were determined after only 2 hours treatment with 72% sulphuric acid at 10° C., and this time of contact has been shown to give somewhat higher results than the 16-hour period finally adopted. Unfortunately, lack of sufficient material prevented repetition of the determinations on the carrot tissues using the latter procedure. Platenius gives no data on the methoxyl content of his lignin residues, hence we are unable to compare the purity of our respective preparations on this basis.

Notable features of the results on the nature of the lignin residues from all these succulent tissues are the extremely low methoxyl and high nitrogen contents. These appear to be characteristics of lignin residues obtained from relatively young and rapidly growing plant tissues. Thus, Phillips *et al.* (10) and Phillips and Goss (9) have recorded somewhat similar values for the nitrogen and methoxyl contents of lignin residues obtained from young oat and barley plants by the 43% hydrochloric acid method. The low methoxyl content of such lignin preparations is no doubt at least partly due to gross contamination of the residues by nitrogenous substances, as is indicated by their high nitrogen contents.

SUMMARY

Pre-treatments with 5% sulphuric and 1% hydrochloric acids prior to determination of lignin were compared using red clover silage, young asparagus shoots, and petioles from mature leaves of the rhubarb plant. Differences in the amount and nature of the lignin residues obtained after these pre-treatments were not consistent.

Three methods for the determination of lignin, the 72% sulphuric acid, the formaldehyde-sulphuric acid, and the holocellulose, were compared using oat straw, clover silage and asparagus shoots. The 72% sulphuric acid procedure yielded the lowest amounts of lignin residues with the highest percentage of methoxyl. The formaldehyde-sulphuric acid method using peptic digestion for the removal of nitrogenous substances gave lignin

residues containing greater absolute amounts of nitrogen than did the 72% sulphuric acid procedure. It was concluded that the higher apparent lignin yields by the formaldehyde method represented greater contamination of the product with non-lignin materials. The higher apparent lignin contents of these materials obtained by the holocellulose determination probably were due to partial removal of protein, and possibly of hemicellulose, along with the lignin. It was concluded that the 72% sulphuric acid procedure was to be preferred.

Some information was obtained on the effect of time and temperature of contact with 72% sulphuric acid on the yield of lignin residues. A 2-hour period of contact of the tissues with the acid at approximately 10° C. was found to give markedly larger residues than 16 hours of contact at this temperature for 6 of the 10 tissues analyzed by both procedures. This evidence of the greater contamination of the lignin residues obtained with the shorter time of contact was supported by the fact that, in 5 of the 7 instances where this information was obtained, the methoxyl contents of the 2-hour residues were lower. The percentage of nitrogen in the lignin residues increased with time of contact with the strong acid in 6 of the 9 instances for which this information is available. However, the absolute amount of nitrogen in the residues remained essentially constant in 3 instances regardless of the time of contact. Two hours contact with the acid at 20° C. gave higher yields of lignin residues than did 16 hours at 10° C. for 3 of the 6 tissues compared, the yields being essentially the same for the remaining three instances. The absolute nitrogen contents of the residues obtained under these conditions were quite similar for 3 of the tissues, somewhat higher for treatment 3 in the remaining 2 instances.

The apparent lignin contents of 7 vegetables, Swede turnips, rhubarb' asparagus, spinach, string beans, carrots, and beets, have been determined using the 72% sulphuric acid method. Boron-deficient turnip tissue was found to have a higher lignin content than normal tissue. Rhubarb petioles and especially beet roots showed a marked increase in apparent lignin content with advancing maturity. However, little or no evidence for a similar increase with age could be found in the results for the asparagus shoots and carrot roots of different stages of maturity.

Notable features of the composition of the lignin residues obtained from all these vegetables were the extremely low methoxyl and high nitrogen contents. The low methoxyl values are probably due, in part at least, to contamination of the lignin residues with nitrogenous substances.

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NOTE

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Further investigation of the effect of methods of preparing succulent plant tissues for the determination of lignin is being carried on in this laboratory by Mr. D. MacDougall (National Research Council bursary grantee) under the direction of W. A. DeLong. In the course of this work it has been found that the temperature of drying such tissues has a marked influence on the amount of apparent lignin obtained. It now appears that the results reported in the above paper, which are for tissues dried at 105° C., represent an over-estimate of the lignin content. A full account of this more recent work will be published later.

THE INHERITANCE OF PLANT COLOUR AND THE EXTENT OF NATURAL CROSSING IN FOXTAIL MILLET[1]

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INTRODUCTION

In the course of the millet breeding work it was found necessary to check on the amount of natural crossing that occurs in the *Setaria italica* species. In order to do this it was necessary to establish the mode of inheritance of some one readily discernible factor. The most obvious one in the material at hand was purple pigmentation which occurs in lines closely related to Empire millet.

It has been well established by Rangaswami (3, 4, 5) and by Kadam (1) that the purple pigmentation condition is a simple dominant to the green condition in *Panicum miliaceum*, *Setaria italica*, and *Eleusine coracana*. The presence of an intensification factor and of one or more factors having to do with the development of the purple pigment has been established, but does not obscure the monogenic dominance of the factor determining the purple condition.

INHERITANCE OF PURPLE COLOUR

Materials and Methods

Certain lines of millet (*Setaria italica*) were found in the breeding nursery to be heterozygous for purple and green plant colour. Progenies from four such heterozygous plants were grown to maturity in the nursery during the summer of 1939. From these F_2 progenies, open-fertilized seed was taken with the knowledge that natural crossing, if it did occur, would be relatively infrequent. F_3 progenies, averaging 150 plants, were grown in pots in the greenhouse and scored for pigmentation while in the seedling stage. By this method a large amount of material was handled in a short time.

Results

Table 1 shows the F_2 segregation of the four F_1 lines.

Table 1.—F_2 segregation for pigmented and green plants

Family	Observed		Calculated		Total	X^2	P
	Pigmented	Green	Pigmented	Green			
1323	54	12	49.50	16.50	66	1.636	.3 to .2
1329	48	17	48.75	16.25	65	.0464	.9 to .8
1258	56	16	54.00	18.00	72	.2963	.7 to .5
1260	44	9	39.75	13.25	53	1.8176	.2 to .1

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It is evident from the data that each of the above families shows good agreement with the expected frequencies of a 3 : 1 ratio.

All the individuals of the F_2 progenies were grown to the seedling stage to give F_3 counts, in order to test further the agreement with the expected 3 : 1 segregation hypothesis. Tables 2 and 3 indicate the results obtained.

TABLE 2.—F_3 SEGREGATION FOR PIGMENTED AND GREEN PLANTS

Family	Observed			Calculated 1 : 2 : 1			Total	X^2	P
	Pure purple	Segregating purple	Green	Pure purple	Segregating purple	Green			
1323	16	38	12	16.50	33.00	16.50	66	2.000	.5 to .3
1329	20	28	17	16.25	32.50	16.25	65	1.2769	.7 to .5
1258	13	43	16	18.00	36.00	18.00	72	2.9712	.3 to .2
1260	16	28	9	13.25	26.50	13.25	53	2.0188	.5 to .3

These data also indicate an agreement with the assumed ratio of 1 : 2 : 1 for all four families.

TABLE 3.—GOODNESS OF FIT OF THE F_3 SEGREGATING PROGENIES

Family	No. of lines	Ave. no. of individuals in each line	No. of lines whose X^2 value is not greater than that of $P = .05\ (N=1)$	No. of lines with X^2 values greater than that of $P = .05\ (N=1)$	Total X^2	P
1329	28	125	28	0	32.253	.3 to .20
1260	28	149	25	3	40.278	.1 to .50
1258	42	115	37	6	53.994	.3 to .20
1323	38	200	31	7	83.943	below .01

The progenies of family 1329 gave a total X^2 value which showed agreement with expectation, while families 1260 and 1258 gave similar results in spite of several progenies in each family showing a poor fit for the expected 3 : 1 ratio. Family 1323 with 31 progenies showing a satisfactory fit with expectation, and 7 progenies at variance with expectation, gave a total X^2 value of 83.943, which shows a real discrepancy. It is probable that the lack of agreement of several of the progenies is due to error in classification. As mentioned previously, the F_3 progenies were only carried to the seedling stage. General observations have indicated that some seedlings which may be classified as green at the early stage, show some pigmentation on reaching maturity. Kadam (1) makes note

of this possibility and records, in working with an F_2 population, that observations in the early stages were misleading since many of the plants recorded showed colour development after full exposure in the field. It has also been mentioned that the F_3 progenies were grown from open-fertilized seed which was subject to some cross-fertilization. The incidence of cross-fertilization might well have a disturbing effect on some segregations since, as will be shown later, some lines are receptive to as much as 10% natural crossing, while other lines seem to be wholly self-fertilized.

On the whole, the preponderance of evidence is in support of the single factor hypothesis in which a main factor P for the antocyanin pigment gives dominance over green.

Observations on the development of the antocyanin pigment are in agreement with those of Rangaswami *et al.* that there are one or more additional factors which condition the degree of intensity of the pigment. These factors, however, do not interfere with the assumption that the presence or absence of pigment is conditioned by the presence or absence of a single dominant factor.

NATURAL CROSSING

The establishment of purple lines of green millet and the knowledge that green is recessive to purple made it possible to get some measure of the amount of natural crossing that occurs in foxtail millet at Ottawa. Early observations had shown the occurrence of natural crossing at Ottawa as high as 4.64% with an average of 2.45%. The following review by Li, Meng, and Liu (2), indicates the results obtained by other workers.

"Li (1934) found the percentage of natural crossing to be 5.60 in a total of 117.627 kernels from plants spaced at a distance of one foot, using non-waxy kernels among waxy seeds as a criterion. Takahashi and Hoshino (1934) found the percentage of natural crossing to be 0.59 as determined by the offspring with coloured stems and leaf sheaths in the non-coloured strains, but in different strains it varied from 0.09 to 1.09. In a series of experiments with mixed sowings of coloured and non-coloured strains these workers obtained up to 2.26% natural crossing."

Li *et al.* in a further check on their material reported a percentage of natural crossing of 7.63, which is fairly close to their previous percentage of 5.60.

Materials and Methods

In order that some definite information might be available on the amount of natural crossing that could be expected at Ottawa, green and purple plants were alternated in an area isolated from other millets. The design was such that 50% of the pollen available for cross-fertilization would be expected to come from the purple plants. It was necessary, therefore, to assume an expectation of double the amount of the crossing that was shown by the progenies of the green plants.

Results

Progenies were grown from a total of 64 plants. As will be seen in Table 4, only 37.5% of the plants showed purple segregates with one plant ranging as high as 4.93% purple. Of a total population of 7,115 seedlings, only 49 were purple, or .689%.

Table 4

Family	Seedings			% Purple
	Green	Purple	Total	
1	84	1	85	1.19
2	92	1	93	1.10
3	92	3	95	3.26
4	76	1	77	1.31
5	71	2	73	2.81
6	99	1	100	1.01
7	95	1	96	1.05
8	83	1	84	1.20
9	81	4	85	4.93
10	64	1	65	1.56
11	58	1	59	1.72
12	151	2	153	1.31
13	108	4	112	3.57
14	123	4	127	3.15
15	58	1	59	1.69
16	160	4	164	2.44
17	150	1	151	.66
18	112	3	115	2.61
19	135	4	139	2.88
20	191	1	192	.52
21	142	2	144	1.39
22	95	3	98	3.06
23	157	2	159	1.26
24	164	1	165	.61
25 to 64	4,425	0	4,425	0

Discussion

It is apparent that while there is a range of natural crossing from 0 in 62.5% of the cases, to 4.93%, or 9.86% if the determined percentage is doubled in accordance with the assumption mentioned above, the average percentage of natural crossing is small. Because of this, and the regulations of the Canadian Seed Growers' Association, which require registered seed growers to return to the breeding institution for foundation stock every two or three years, it is evident that natural crossing will have little or no effect on maintaining the purity of seed stocks of millet.

Summary

1. Evidence is presented to support the hypothesis that purple pigment represented by a single main factor is dominant over green, and that inheritance is on a monogenic basis.

2. An average of .689% natural crossing was found by testing the progenies of 64 pure green lines exposed to purple pollen. A range of natural crossing from 0 to 9.86% was established.

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INVESTIGATIONS CONCERNING THE COUMARIN CONTENT OF SWEET CLOVER[1]

III. The Inheritance of the Low Coumarin Character

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The development of a variety of *Melilotus alba* having a low coumarin content has been described by Stevenson and White (6). They reported that this variety was being named "Pioneer" and that its foliage contained about one-tenth the quantity of coumarin found in unselected sweet clover. Based upon the coumarin determinations of about 200 F_2 plants in their first year of growth, the same authors tentatively suggested that the low coumarin character was inherited as a simple recessive. The purpose of this paper is to present more detailed evidence of the inheritance of the low coumarin character, based upon the analysis of data from the F_1 to F_3 populations.

MATERIALS AND METHODS

Determinations of percentage coumarin in the foliage were made by the method originally described by Clayton and Larmour (2), but modified slightly as outlined by Stevenson and White (6). A discussion of the extent and sources of variations in using this test has been presented by White and Horner (7).

In 1938 strains of the Pioneer variety were crossed in the field with each of the following sweet clovers, all high in coumarin:

(*a*) A strain similar in parentage and type to the Pioneer strains;
(*b*) A vigorous growing, late maturing strain (S-268);
(*c*) The dwarf, finely branching Alpha variety;
(*d*) A white sepal strain of the Alpha variety.

The low coumarin plants were used as female parents in some crosses and as males in others. The technique of crossing consisted of selecting about 10 newly opened and untripped flowers on each raceme, removing all the petals, blowing strongly on the stigma to remove pollen, applying the male pollen immediately with a toothpick, and covering with a glassine bag. This technique usually gives a high proportion of crosses, but since an absolute emasculation is not performed some selfs usually occur.

The F_1 of the above crosses were grown in bins in the greenhouse during the winter of 1938-39, and tested at the early flowering stage for coumarin content.

In the spring of 1939 the F_2 were started in the greenhouse in flats and transplanted to the field in early June. In the same fall a proportion of these F_2 plants were tested and the results reported by Stevenson and

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White (6). In the following spring over 1200 of these plants were tested. Tests were made at as near the flowering stage as possible, but because of the large population testing extended from late budding to early seed setting stages.

The F_3 generation consisted of selfed progenies of 52 F_2 plants and was sown in the greenhouse in September, 1940. One flat containing 24 seeds was sown to progenies of each of 34 F_2 plants which tested between .075 and .7% coumarin. The progenies of low testing F_2 plants were expected to be homozygous. Therefore it was considered that if 5 or 6 F_3 plants were grown from each low testing F_2 parent and these F_3 plants all tested low, the chances that they were samples of a segregating line would be very small. Accordingly, only 8 seeds of each of 18 low testing F_2 parents were sown with 3 progenies in each flat. These plants were tested for coumarin content about three months after seeding when the most advanced plants were just beginning to flower.

Throughout this paper the coumarin content is expressed as a percentage of green weight.

EXPERIMENTAL RESULTS

The First Generation

The data on the coumarin content of the F_1 are presented in Table 1 in the form of a frequency distribution.

TABLE 1.—FREQUENCY DISTRIBUTION OF F_1 PLANTS FOR COUMARIN CONTENT EXPRESSED AS PERCENTAGE OF GREEN WEIGHT

Coumarin content of parents		No. of F_1 plants with coumarin content of										
Female	Male	.00	.01	.02	.03	.04	.05	.06–.09	.10–.13	.14–.17	.18–.24	.25+
.00	.20–.35	7	3	4	4	3	6	10	14	10	7	7
.10–.15	.00			1		1		2	7	4	6	6
.25–.30	.00							3	5	2	2	

As will be shown by data to be presented later, those plants having a coumarin content of .10 or over may be classified as having a high coumarin content. Considering the F_1 of crosses of low coumarin (.00) × high coumarin, 27 plants were low, 10 plants were intermediate, and 38 were of high coumarin content. In the light of the results of the reciprocal crosses, the 27 low testing plants (.00 to .05) were undoubtedly selfs. The 10 plants giving an intermediate test should have been retested to permit of proper classification. The 38 high testing plants were undoubtedly hybrids and they were used as the parents of the F_2. In the progenies of the reciprocal crosses (high × low) only two F_1 plants tested low, and the remainder are classified as high testing. Unfortunately the two low testing plants were not retested, and their proper category is in doubt. Of the high testing plants in the high × low crosses, about two-thirds were of the same general range or lower than the high parent. It was impossible to distinguish hybrids from selfs in this group.

On account of the fairly large proportion of F_1 plants which tested somewhat lower than the high coumarin parent, Stevenson and White (6) described the F_1 as "more or less intermediate in coumarin content." In view of the discussion above and the data to follow on the segregating generations, it is now considered that the high coumarin character is definitely dominant, and thus the F_1 is more properly described as having a high coumarin content.

The Second Generation

As pointed out above, the high testing F_1 plants from the low $\times$ high crosses were used as the parental material for the F_2. A large proportion of the population was tested for coumarin content in 1940, the results of which are presented in Table 2.

In Table 2 the families are listed in the order in which they were tested. Those tested first were sampled in the late bud to early flowering stage, but in the last families tested the plants were in the early stages of seed setting. It is evident that there was a tendency for a decrease in percentage coumarin and a greater proportion of intermediate types as flower-

TABLE 2.—FREQUENCY DISTRIBUTION OF F_2 PLANTS FOR COUMARIN CONTENT EXPRESSED AS A PERCENTAGE OF GREEN WEIGHT

*Group	Family No.	No. of plants with coumarin content of										
		.00–.01	.02–.03	.04–.05	.06–.09	.10–.14	.15–.19	.20–.29	.30	.40	.50	.60+
Group I	C-38-I- 5-1	25	3	—	—	4	5	16	18	19	9	4
	" -21-1	9	15	4	1	—	2	6	5	22	17	25
	" -32-1	19	3	1	—	—	1	—	6	12	14	21
	" -52-1	9	2	4	1	1	—	11	18	10	4	31
	Totals	62	23	9	2	5	8	33	47	63	44	81
Group II	C-38-I-40-1	22	9	10	—	1	1	2	23	14	17	3
	" -47-1	15	4	1	3	1	1	2	13	6	9	17
	" -72-1	23	4	1	1	2	7	15	16	5	4	9
	" -80-1	14	6	2	3	5	9	22	18	20	10	2
	" -89-1	11	5	7	6	6	11	10	8	7	17	10
	Totals	85	28	21	13	15	29	51	78	52	57	41
Group III	C-38-III- 2-1	7	12	4	3	1	9	24	21	3	4	2
	" -15-1	3	6	4	2	4	8	15	15	16	5	5
	" -25-1	4	4	1	—	4	9	20	11	2	—	—
	" -28-1	7	13	11	7	9	8	9	16	3	—	—
	" -33-1	9	4	3	3	7	6	17	2	—	1	—
	Totals	30	39	23	15	25	40	85	65	24	10	7
Parents	Pioneer lines	120	18	13	—	3	1	—	—	—	—	—
	S-34-40-1-1	—	—	—	—	—	—	1	7	2	3	7
	Alpha 2	—	—	—	—	—	—	1	—	1	2	7
	White Sepal Alpha	—	—	—	—	1	—	4	6	3	—	—

* Group I—Crosses of low coumarin with strains of common white.
Group II—Crosses of low coumarin with Alpha sweet clover.
Group III—Crosses of low coumarin with white sepal Alpha.

ing progressed. White and Horner (7) have previously shown this same tendency. In spite of this variability there is a fairly clear cut bimodal distribution of plants, the one group being of low and the other of high coumarin content.

The selection of a level to divide low from high coumarin tests must be somewhat arbitrary. As will be shown in the presentation of F_3 data, those F_2 plants testing .00 to .05% usually gave rise to progeny with coumarin tests falling in the same range, whereas F_2 plants testing .10% or more gave rise to progenies either homozygous for readings of .10% or more or segregating for high and low tests. It therefore seems reasonable to classify those F_2 plants testing .05% or less as low and those testing .10% or more as high. The proper designation of the small proportion of the population which fell in the .06–.09% class is less evident. The retesting of a number of plants in this class indicated that approximately equal numbers belonged to the high and low groups. F_3 progenies of two F_2 plants testing .075% showed that they were actually low testing plants. Because of the tendency for coumarin content to decline as the flowering progressed, it was considered that those plants which were not retested in the .06–.09% class of Groups II and III of Table 2 were most properly classified in the high group. The two plants in Group I which tested .06–.09% were classified as low.

On the above basis the F_2 plants were classified as low or high coumarin, and the data are presented in Table 3, along with the X^2 and P values for the goodness of fit analysis of the data to a single factor hypothesis.

TABLE 3.—SEGREGATION OF F_2 POPULATION FOR COUMARIN CONTENT AND X^2 AND P VALUES FOR GOODNESS OF FIT TO A 3 : 1 RATIO

Group	No. of low coumarin plants	No. of high coumarin plants	Value of X^2 for fit to a 3 : 1 ratio	P value of X^2
I	96	281	.0508	.8
II	134	336	3.0893	.07
III	92	271	.0229	.9
Total	322	888	n = 3, P = .4; n = 1, P = .2	

From Table 3 it is seen that the observed segregation does not deviate significantly from the assumed 3 : 1 ratio, either in the individual groups or on the basis of the entire population. In Group II the P value bordered on significance, and it is possible that if the plants in the .06–.09 coumarin class had been classified differently there may have been a significant deviation of the observed from the expected ratio for that group. However, the data definitely appears to warrant the conclusion that the low coumarin character is inherited as a simple recessive. Stevenson and White (6) reached a similar conclusion based on the analyses of around 200 plants of the above population in their first year of growth.

Two of the high coumarin parents of the crosses were of the Alpha type, and in addition one of these Alpha type parents had a white sepal character. The Alpha character has been shown to behave as a simple recessive by Kirk (4), Stevenson (5), Elders (3) and Clarke (1). The inheritance of the white sepal character has as yet not been reported. It is interesting to note that in the present investigation there was no evidence of linkage between the low coumarin character and either the Alpha or white sepal character.

Although not bearing directly on the inheritance of coumarin content, it is a matter of general interest to examine the correlation between the tests for coumarin content of the first and second years' growth of the F_2. At the flowering stage of the second year's growth 291 F_2 plants were tested which had been tested in early September of the year previous. The 2-years' data on these plants are given as a correlation surface in Table 4.

TABLE 4.—CORRELATION SURFACE FOR COUMARIN CONTENT OF FIRST AND SECOND YEARS' GROWTH OF 291 F_2 PLANTS

Coumarin content of 1st yr. growth (percentage green weight)	Coumarin content of 2nd year growth (percentage green weight)														
	.00	.01	.02	.03	.04	.05	.075	.10	.20	.30	.40	.50	.60	.70	Totals
.00	26	1	1	2	—	—	1	—	—	—	—	—	—	—	31
.01	6	—	1	2	1	1	—	—	—	—	—	—	—	—	11
.02	6	—	—	1	—	—	—	—	—	—	—	—	—	—	7
.03	2	1	5	1	3	2	—	—	—	—	—	—	—	—	14
.04	—	—	3	2	—	2	—	—	—	—	—	—	—	—	7
.05	4	—	1	1	—	—	—	—	—	—	—	—	—	—	6
.075	—	—	1	1	—	—	—	—	—	—	—	—	—	—	2
.10	1	2	1	—	—	—	—	2	—	1	—	—	—	—	7
.20	—	—	—	—	—	—	—	1	1	1	5	2	4	3	17
.30	—	—	—	—	—	—	—	—	5	10	13	12	7	1	48
.40	—	—	—	—	—	—	—	3	7	12	13	9	8	3	55
.50	—	—	—	—	—	—	—	2	5	8	12	6	3	3	39
.60	—	—	—	—	—	1	—	2	5	8	13	1	9	—	39
.70	—	—	—	—	—	—	—	1	1	4	1	—	1	—	8
Totals	45	4	13	10	4	6	1	11	24	44	57	30	32	10	291

From Table 4 it is seen that with the exception of 5 plants, from the first to the second year there was a perfect agreement between the classification of plants as having low or high coumarin content. Plants which tested low in their first year were low in their second year, likewise for high coumarin plants. This clear-cut grouping is responsible for the fact that the correlation was found to be +.87 which is a highly significant value. In a similar study with non-hybrid material, White and Horner (7) obtained a very similar correlation value. No explanation can be offered for the fact that 5 plants were classified in one group the first year and the other group the second year.

While between groups there is a high correlation, there is little or no evidence of correlation within either the high or low groups. In a population of low testing, non-hybrid plants, White and Horner (7) likewise demonstrated a lack of significant correlation of one year's tests with the next.

The Third Generation

In order to determine the breeding behaviour of F_2 plants classified as having low, intermediate and high coumarin content, an F_3 population was studied. The results from the tests on a portion of the F_3 plants have already been discussed in the previous section, but the complete data are presented in Table 5.

TABLE 5.—FREQUENCY DISTRIBUTION OF F_3 PLANTS FOR COUMARIN CONTENT EXPRESSED AS A PERCENTAGE OF GREEN WEIGHT

No. of F_3 families	Test of F_2 parents	No. of F_3 plants with coumarin content of									
		.00–.01	.02–.03	.04–.05	.06–.09	.10–.14	.15–.19	.20–.29	.30	.40	.50+
17	.00–.05	104	13	4	—	—	1	1	—	—	—
1	.00	—	—	—	—	2	2	4	—	—	—
2	.075	30	8	3	—	1	—	—	—	—	—
9	.30–.70	—	—	—	—	35	37	45	54	20	5
22	.10–.70	50	36	19	7	46	67	97	86	40	19
1	.70	18	3	—	—	—	—	—	—	—	—
Parent—Pioneer		10	7	2	—	—	—	—	—	—	—
" —Arctic		—	—	—	—	2	7	7	3	1	—

In 15 of the 18 progenies of low testing F_2 plants all the F_3 plants tested .05% or lower. In each of two such progenies, however, one plant appeared which showed a substantial amount of coumarin. It seems reasonable to assume that these two high testing F_3 plants in otherwise low testing progenies were either volunteers or hybrids resulting from accidental crosses. As shown in Table 5, one of the low testing F_2 plants gave rise to a high testing progeny. In this case it appears that there was an error in the numbering of a plant in the field or in the recording of a test. The data from 17 of the 18 progenies of low testing F_2 plants seem to justify the conclusion that plants testing between .00 and .05% are homozygous for the recessive low coumarin character.

Progenies of only two intermediate testing (.075%) F_2 plants were included in the F_3. As shown in Table 5, these two F_2 plants gave rise to true breeding, low testing progenies. If progenies of a larger number of plants of this type had been studied, it seems likely that a portion of them would have shown high or segregating tests, since, as previously mentioned, retesting plants of this intermediate group shows that about equal proportions may be reclassified as low or high.

One F_2 plant which had tested .70% gave rise to a low testing progeny, also indicating an error in numbering or recording. Of the remaining 31 progenies of high testing F_2 plants, 9 proved to be true breeding for high coumarin content and 22 were segregating. On the basis of a single factor hypothesis, this agrees very well with the expected frequency of 1

homozygous high line to 2 segregating lines, the P value being .6. In the 22 segregating lines, 362 plants tested .075% coumarin or more, and 105 plants tested .05% or less, which also agrees with the expected 1 : 3 ratio, the P value being .2.

The tests on the F_3 thus substantiate the classification of plants having .05% coumarin or less as low, and those having .10% coumarin or more as high. The proper designation of the small proportion of plants testing .06 to .09 is doubtful, and it would appear that such tests should be repeated. On the basis of the tests on the F_3, it may be concluded that low coumarin F_2 plants are true breeding, and that of the high testing F_2 plants one-third are homozygous high, and two-thirds are heterozygous, segregating for high and low coumarin in the ratio of 3 : 1.

DISCUSSION

While the segregation in the F_2 and F_3 clearly show that the low coumarin character is inherited as a simple recessive, the results indicate that plants may display a considerable range in coumarin content and still be of the same genetic constitution. In Table 4, for example, it has been pointed out that within the group of low testing and also the high testing plants there was little or no evidence of correlation of the test of the first year with that of the second. Plants testing .00% one year might test anywhere from .00 to .05 the second year, and those testing .10 might test anywhere from .10 to .70% the next year. Also the F_2 parents of 10 of the 22 segregating F_3 progenies (Table 5) tested between .10 and .18% coumarin, while the remaining 12 F_2 parents tested between .60 and .70% but no difference could be detected in the distribution of the F_3 progenies. On the surface it may be considered significant that the 9 F_2 parents which gave rise to homozygous high testing F_3 families (Table 5) all contained coumarin in excess of .30%, and 7 of the 9 contained .60 to .70% coumarin, while of the F_2 parents which gave rise to segregating progenies almost one-half (10 out of 22) tested less than .20%. However, over one-third of the plants in the progenies of the homozygous high testing F_2 parents fell in the lower range (.10–.19) of the distribution for high testing plants. The evidence so far available indicates that there is no genetic basis for the variations in coumarin content of over .10% or under .05%.

SUMMARY

1. Data on the coumarin content of the F_1, F_2 and F_3 of crosses between low and high coumarin lines and varieties are presented.

2. From the F_1 it was shown that high coumarin content was dominant.

3. The segregation of 1210 F_2 plants showed a good fit to a 3 : 1 ratio. No linkage was observed between coumarin content and "Alpha" habit of growth or white sepals.

4. The low coumarin F_2 plants proved to be true breeding. Nine high testing F_2 plants were true breeding for high coumarin content, while 22 F_2 plants were found to be segregating in the F_3. This ratio of pure breeding to segregating lines closely fits the expected 1 : 2 ratio.

5. It is concluded that the low coumarin character is inherited as a simple recessive.

ACKNOWLEDGMENTS

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A STUDY OF THE CHLOROPHYLL, XANTHOPHYLL AND CAROTENE CONTENTS OF THE WHEAT KERNEL HARVESTED AT SUCCESSIVE STAGES OF DEVELOPMENT[1]

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The Division of Chemistry has long been interested in the relation of stage of maturity and quality of wheat to its chemical composition. Earlier studies were confined particularly to the protein and ash contents.

In recent years a quantity of western wheat has been reduced in grade because of a greenish cast. Little is known about the changes that take place in the pigment content of the wheat kernel at different maturation stages. It was thought that a study of the changes in the chlorophyll, xanthophyll, and carotene contents of wheat with advancing maturity might yield valuable information. This paper presents the results of this study, a description of a modified method for the determination of chlorophyll, and an investigation of the chemical properties of an unidentified brown colour.

LITERATURE REVIEW

The carotenoid pigment content of wheat flour is a factor considered in commercial milling and flour bleaching practice. It has been given careful consideration by cereal chemists and plant breeders. Monier-Williams (6), after working with petroleum ether extracts of flour, came to the conclusion that the colouring matter was either identical with, or closely related to, carotene. Palmer (11) noted the presence of carotene and xanthophyll in wheat. Ferrari and Bailey (1) confirmed the work of Monier-Williams. Their evidence tended to establish the major pigment of flour extract as carotene, with little, if any, xanthophyll.

Markley and Bailey (5) used the Willstätter and Stoll (15) method of separating carotene from xanthophyll. This method is based on the preferential solubility in an immiscible naphtha-methanol mixture. Their analyses of 4 samples of wheat showed 12.8 to 34.8% of the total pigment to be carotene. They also obtained absorption curves of the naphtha (carotene) and methanol (xanthophyll) fractions, but did not draw any definite conclusions as to the nature of the pigments.

Munsey (7) used a hot saponification process similar to that of Guilbert (2), and concluded that the main portion of the flour pigment was xanthophyll or a closely related pigment, and that previous high results for carotene were obtained because xanthophyll esters were not properly saponified and were found in the petroleum spirit layer. He claimed that, although the total pigment amounted to from 2 to 3 p.p.m., the carotene amounted to not more than 0.2 p.p.m.

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Seaber (13) stated: "Experiments on a few flours with our 3% acetone process confirm Munsey's statement that the amount of B-carotene is very small (probably of the order of 0.15%), but in the particular flours examined I found very little xanthophyll after either hot or cold saponification."

Although the carotenoid pigments have received much attention from cereal chemists, the author did not find any reference in the literature to the changes that take place in the carotenoid pigment content of wheat during ripening. Most of the available data did not differentiate between carotene and xanthophyll contents.

In so far as can be ascertained, chlorophyll has not heretofore been determined in whole wheat meal.

STUDIES IN 1939

Experimental

One thousand heads of Regent wheat were harvested on 10 different dates, approximately 4 days apart, in 1939. The first samples were harvested about 2 weeks after heading while the kernels were very green, and the last about 2 weeks after the normal time of harvesting. The heads were dried at 100° C. for 1 hour and at 70° C. for 36 hours, after which the kernels were dry and hard. The purpose of drying the samples was to stop all enzyme action as soon as possible and to facilitate threshing. After threshing, the samples were ground in a Wiley mill to pass a No. 16 sieve and stored in the dark in glass bottles with screw caps.

Carotenoid Pigments

The method of Guilbert (2) was used to determine carotene and xanthophyll. A 15 g. sample of whole wheat meal was refluxed for 30 minutes with 100 ml. of a saturated solution of potassium hydroxide in ethyl alcohol and then extracted with ethyl ether. The ether was distilled under reduced pressure and the residue containing the carotenoid pigments was fractionated between petroleum ether and 85% methyl alcohol. Measurements were made with an Evelyn photoelectric colorimeter equipped with a 440 filter which transmitted light of wavelengths between 4100 Å and 4750 Å. A standard curve was prepared using B-carotene. The same curve was used to determine the xanthophyll in the methyl alcohol phase. The results of these analyses are given in Table 1.

Chlorophyll

The method used for chlorophyll determinations was essentially that of Willstätter and Stoll with some modifications as outlined by Oltman (10) and some further variations introduced by the author.

The method as outlined by Oltman was briefly as follows. A weighed quantity of material was extracted in a Soxhlet extractor with a mixture of equal proportions of ether and acetone for 24 hours on a steam bath below 50° C. The extract was placed in a separatory funnel and the acetone was washed out with water. The flavones and anthocyanins were washed out with 1% sodium carbonate. The solution was washed twice further with distilled water. Ten ml. of a saturated solution of potassium hydroxide in methyl alcohol were added and the solution was placed in the

refrigerator over night. The green potassium chlorophyllin was separated from the carotenoids by adding at least 10 ml. of water. The alcohol phase was drawn off, washed once with ethyl ether, made up to volume and measured in a photoelectric colorimeter.

Measurements were made with an Evelyn photoelectric colorimeter using a 660 filter which transmits light of wavelengths between 6350 Å and 7200 Å. A standard curve was made using pure chlorophyll obtained from the Eimer and Amend Co. A 0.0163-gm. sample of chlorophyll, which had been dried in a vacuum desiccator over sulphuric acid for one week, was weighed out and dissolved in 100 ml. of ethyl ether. To the solution were added 10 ml. of a saturated solution of potassium hydroxide in methyl alcohol and the solution was placed in the refrigerator over night. The potassium chlorophyllin which was formed was green in colour and soluble in water. The ether was washed several times with water and the combined washings were made up to 250 ml. This was taken as a stock solution and dilutions were made from it.

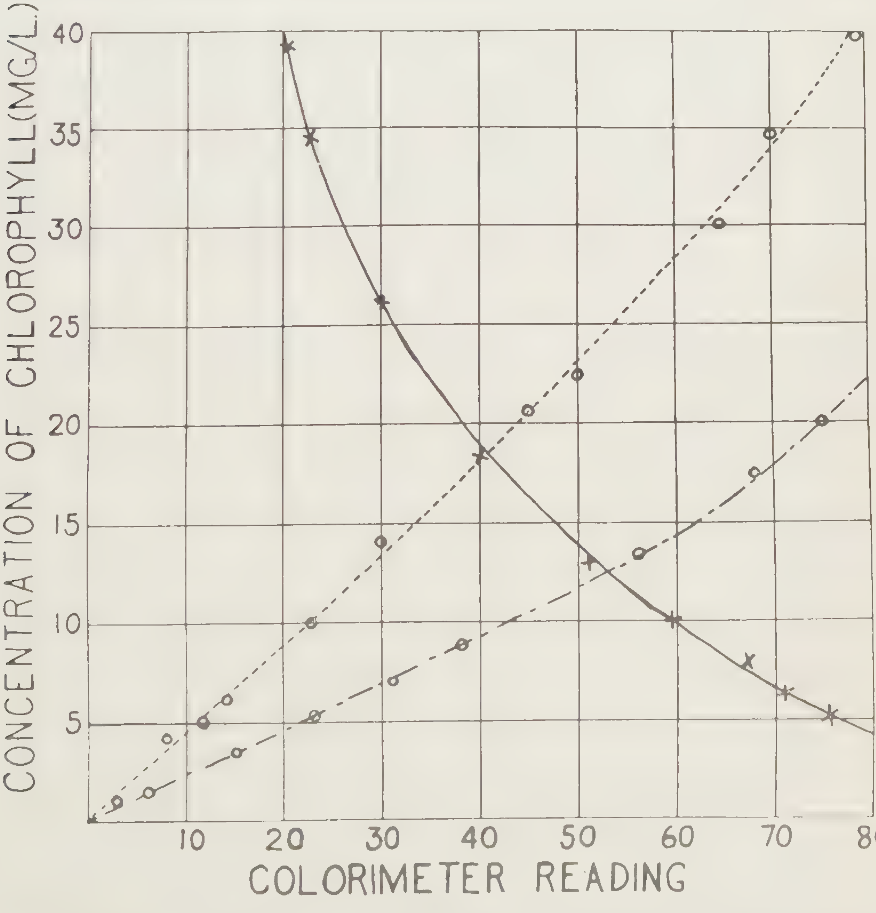

FIGURE 1. Calibration curves of chlorophyll. *Solid line.* Potassium chlorophyllin in alcohol for the Evelyn photoelectric colorimeter. *Dotted line.* Potassium chlorophyllin in alcohol for the Klett-Summerson photoelectric colorimeter. *Dot and dash line.* Chlorophyll in acetone for the Klett-Summerson photoelectric colorimeter.

On following this procedure using a 25-gm. sample of whole wheat meal from cut VI, it was noted that a brown colour developed in the solution after the addition of alcoholic potassium hydroxide. When the water was subsequently added and the separatory funnel shaken gently, a brown ring separated out between the two phases. On more vigorous shaking the brown colour passed into the alcohol phase. However, when the alcohol phase was drawn off and shaken vigorously with ether, the brown colour passed into the ether. When the ether phase was washed with 1% sodium carbonate, the brown colour was transferred to the sodium carbonate phase and so it was thought that more careful washing with 1% sodium carbonate would overcome the difficulty. The brown colour, however, appeared again.

It was necessary to find some way of getting rid of the substance responsible for the brown colour because it entered the alcohol phase and interfered with the measurement of the potassium chlorophyllin.

Guthrie (3) determined the relative amounts of a brown pigment in leaves which was soluble in 30% acetone. Neish (8) removed an interfering brown pigment from leaves by extraction with water. It was thought that the brown substance in the wheat extracts might be this pigment. Several experiments were carried out with a view to removing the substance from the whole wheat meal by extraction with 30% acetone or water before carrying out the chlorophyll determination. Both 30% acetone and water failed to remove the substance responsible for the brown colour.

Attempts to eliminate the brown colour by washing the ether solution of pigments with 25% methyl alcohol proved unsatisfactory. It had been previously determined that 25% methyl alcohol does not remove chlorophyll from ethyl ether solution. This indicated that the solubility of the substance was affected by treatment with alcoholic potassium hydroxide solution.

A chromatogram was prepared using a mixture of Fuller's earth and magnesium oxide in equal proportions as an adsorbent. The pigments were dissolved in petroleum ether containing 20% ethyl ether. The substance responsible for the brown colour was adsorbed in the same zone as the pigments. It was concluded that the separation of chlorophyll from this substance by means of a chromatogram would be very difficult.

The brown colour was insoluble in carbon disulphide, benzene, and petroleum ether.

To determine whether or not the brown colour was formed during saponification by the oxidation of some substance in the extract, saponification of an extract was carried out in an atmosphere of nitrogen. The brown colour appeared as before.

Another possibility was that the brown colour might have been formed from decomposition products of chlorophyll. Chlorophyll was added to a 25-gm. sample of patent flour and the procedure carried out along with a control. The brown colour was formed in both cases. The substance responsible for the brown colour was therefore present in patent flour.

It is known that carbohydrates will turn brown in potassium hydroxide solution, so a Molisch test was carried out on some of the brown solution and a positive test was obtained.

Since carbohydrates are not thought to be soluble in anhydrous ether, a number of experiments were carried out to separate the pigments from the substance responsible for the brown colour by dissolving the former in anhydrous ethyl ether. Anhydrous petroleum ether and benzene were also used, but the brown colour persisted in developing after the addition of the alcoholic potassium hydroxide solution. It was concluded that the substance responsible for the brown colour was soluble in anhydrous ethyl ether, petroleum ether, and benzene. Although a positive Molisch test was obtained, it seemed very unlikely that the substance was carbohydrate in nature.

Some proteins give a positive Molisch test, so a biuret test was carried out on the brown solution and a slight violet colour noted. Since the solution being tested was already coloured brown, it was very difficult to detect the presence of any violet colour and later attempts were unsatisfactory. It was found that when an aqueous solution of the brown substance was saturated with ammonium sulphate and washed with ether, all of the brown colour passed into the ether. In an alcoholic potassium hydroxide solution a very heavy white precipitate of potassium sulphate was formed. Tannic acid and sodium chloride were of little value. Phosphotungstic acid was effective but ammonium chloride was the most satisfactory reagent to force the brown colour from an alcohol phase to an ether phase.

The colour intensity of a 50% alcoholic potassium hydroxide solution of potassium chlorophyllin was measured in the colorimeter. The solution was then saturated with ammonium chloride and washed with ethyl ether. The solution after treatment gave exactly the same reading as before, so ammonium chloride did not affect the solubility of potassium chlorophyllin.

Steche (14), in his modification of Willstätter's method, removed the acetone from the extract by washing 5 times with 100 ml. of 1% potassium chloride solution and once with 100 ml. of 3% potassium chloride solution. This procedure was followed and the results were in good agreement with the Oltman (10) method and there was much less difficulty with emulsions. It was therefore adopted.

A known amount of chlorophyll was added to a sample that had been analysed and good recovery was obtained.

Several experiments were carried out to determine the number of saponifications necessary to convert all the chlorophyll to potassium chlorophyllin and one was found to be sufficient.

Procedure for Determining Chlorophyll in Whole Wheat Meal

A weighed quantity of whole wheat meal was extracted in a Soxhlet extractor with a 1 to 1 ether/acetone mixture on a steam bath for 24 hours or until no further colour was noted. The extract was placed in a separatory funnel and the acetone removed by washing 5 times with 100 ml. of 1% potassium chloride and once with 100 ml. of 3% potassium chloride solution. (The potassium chloride solutions contained 7% ethyl ether.)

The ethyl ether solution of the pigments was placed in a 125 ml. erlenmeyer flask and 10 ml. of methyl alcohol saturated with potassium hydroxide were added and the solution left in the refrigerator over night. At least 10 ml. of water were added and the lower greenish layer containing the potassium chlorophyllin was drawn off. This solution was saturated with ammonium chloride and washed once with ethyl ether. The alcoholic solution of potassium chlorophyllin was diluted to a convenient volume and the colour intensity measured in a photoelectric colorimeter, using a filter which transmitted only light of wavelengths above 6350 Å.

The results of analyses of 10 samples of Regent wheat cut on different dates are given in Table 1. Since all of the data were calculated on a dry matter basis, the results expressed in micrograms per 1000 kernels gave the better picture of the changes which took place in the pigment content of the wheat kernel as it matured.

TABLE 1.—RELATION OF STAGE OF RIPENING TO THE CAROTENE, XANTHOPHYLL AND CHLOROPHYLL CONTENTS OF WHOLE WHEAT MEAL (1939)

No. of cut	Date of cutting	Dry Matter Basis						
		Carotene	Xanthophyll	Chlorophyll	Wt. per 1000 kernels	μg. per 1000 kernels		
						Carotene	Xanthophyll	Chlorophyll
		p.p.m.	p.p.m.	p.p.m.	gm.			
1	July 20	.891	11.60	267.5	18.7	16.6	217	5002
2	24	.674	8.73	173.2	26.4	17.8	230	4572
3	27	.426	5.64	99.2	32.5	13.8	183	3224
4	31	.128	3.72	43.0	38.2	4.9	142	1643
5	Aug. 3	.212	3.79	38.0	34.5	7.3	131	1311
6	7	.120	2.50	2.3	38.6	4.6	96	89
7	10	.139	2.56	*	39.2	5.4	100	—
8	14	.143	2.50	—	36.2	5.2	90	—
9	17	.175	2.07	—	41.9	7.3	87	—
10	21	.231	2.07	—	38.8	9.0	80	—

The wheat was mature on Aug. 7.
* Less than 1 p.p.m.

It will be seen from Table 1 that the carotene, xanthophyll and chlorophyll contents decreased as the wheat matured. Xanthophyll was the major carotenoid pigment of the wheat kernel. The chloroplast pigments reached a minimum very close to the normal date of harvesting. The rapid decrease in the chlorophyll content near this stage was very significant. When wheat was cut a few days early, the chlorophyll content was relatively high.

Petering, Wolman and Hibbard Method for Determining Chlorophyll

Since these analyses were completed, Petering, Wolman and Hibbard (12) have published a method for determining chlorophyll in plant tissue, without separating the pigments.

This procedure was applied to whole wheat meal and was found to give results in good agreement with the method herein described, provided the chlorophyll content of the meal was relatively high. When the

chlorophyll content was low the results by this method were much higher than those previously obtained. A Klett-Summerson photoelectric colorimeter equipped with a 66 filter which transmitted light of wavelengths only above 6400 Å was used. The results of several determinations are tabulated in Table 2.

TABLE 2.—COMPARISON OF TWO METHODS FOR DETERMINING CHLOROPHYLL IN WHOLE WHEAT MEAL

Nature of sample	Weight of sample	Chlorophyll	
		Petering Method	Writer's Method
	gm.	p.p.m.	p.p.m.
Cut IV	5	62.5	62.5
		60.0	55.0
Cut V	10	40.0	41.0
		46.3	45.0
Cut VI	50	7.0	2.5
		7.0	3.7
Normal bran	25	4.5	1.0
Normal wheat	100	1.5	0.8
Normal bran	50	5.5	1.5
Normal wheat	100	1.2	0.8

Apparently there was present in whole wheat meal, and more especially in bran, some pigment other than chlorophyll which had an absorption band above 6400 Å. It did not seem advisable to determine chlorophyll in whole wheat meal unless it had been separated from the other pigments.

STUDIES IN 1940

Willstätter (15) and Oltman (10) dried green plant material for chlorophyll determinations at 40° C. Green kernels of wheat were dried at this temperature and the green colour disappeared very rapidly.

Five samples of Regent wheat were harvested on successive dates. The first 2 samples were harvested when the kernels were very green and immature, the third at the normal time of harvesting and the last 2 after normal harvesting. Immediately after harvesting the heads were placed in a cold storage chamber at −20° F. to stop all enzyme action. While frozen, the heads were threshed by pounding in a bag. The frozen kernels separated quite readily from the chaff. The kernels were stored over concentrated sulphuric acid in a desiccator at −20° F. After 2 months the first 2 samples contained about 40% moisture.

To determine the effect of drying by heat on the chlorophyll content of wheat kernels, 2 approximately 5-gram portions of sample 2 were subjected to different treatments. The first was ground with sand in a mortar and then extracted in a Soxhlet apparatus with U.S.P. acetone until no further colour was removed. An equal volume of ether was added and the procedure for the determination of chlorophyll as given above was followed. The second lot was dried at 100° C. in a vacuum oven for 5 hours, ground with sand and extracted. There was no significant difference between the chlorophyll contents of the 2 portions. Similar results were

obtained when a 100° C. oven with forced draft ventilation was used. Drying wheat kernels at 100° C. did not cause any detectable loss in chlorophyll content.

The 5 samples were dried in a 100° oven with forced draft ventilation for about 3 hours. After drying, they were ground in a hammer mill to pass a No. 20 sieve and stored in the dark in glass bottles with screw caps. Chlorophyll determinations were carried out as above using acetone to extract the pigments.

The chlorophyll contents of the 5 samples of wheat harvested at successive stages of maturity are reported in Table 3.

Table 3.—Relation of stage of ripening to the chlorophyll content of whole wheat meal (1940)

No. of sample	Date of cutting	Dry matter basis		
		Chlorophyll	Wt. per 1000 kernels	μg. of chlorophyll per 1000 kernels
		p.p.m.	gm.	
1	Aug. 6	157.2	30.5	4794
2	9	105.7	31.0	3277
3	13	1.0	31.5	32
4	14	1.9	31.7	60
5	16	1.9	27.2	52

The wheat was mature on Aug. 13.

As was noted with the 1939 crop the chlorophyll content decreased as the wheat matured. The fact that the loss of chlorophyll during the 4 days preceding the date on which the wheat was mature was greater in 1940 than in 1939 may be accounted for by the high temperatures that prevailed during this period. The samples were not harvested from randomized plots so the slight increase in chlorophyll content of samples 4 and 5 was probably due to sampling error.

The Brown Colour

Kent-Jones and Herd (4) presented a method for determining the quantity of a reddish-brown pigment in flour. They considered that it gave a measure of the amount of red bran pigment contaminating the sample of flour under examination. The pigment was extracted with alkaline methyl alcohol. The only property mentioned was that it was almost colourless in acid solution and brownish-yellow in alkaline solution.

Markley and Bailey (5) found that their 67% alcohol extracts of whole wheat meal were yellow, with a slight brownish tint, in acid solution and intense greenish-yellow in alkaline solution. Upon standing a few days the alkaline solution became dark brownish-yellow. An alkaline acetone extract of ground Marquis wheat, which had been previously successively extracted with gasoline, neutral acetone and 0.4% NaOH in 67% aqueous acetone, after standing in the dark for 2 years at room temperature was dark red in colour. This extract was decanted and ether added until there was a distinct layering. The water layer contained a very dark red

colour, which was insoluble in carbon bisulphide. This dark red extract reacted positively with the Folin and Denis reagent, but had no pronounced indicator properties and was not precipitated by acids.

Experimental

An aqueous solution of the brown substance was found to give the greatest galvanometer deflection when a 440 filter was used in the Evelyn photoelectric colorimeter. Dilutions were made from a concentrated solution and it was found that the solution obeyed Beer's law. A standard curve was prepared. Using this arbitrary standard curve, the amount of the substance responsible for the brown colour was determined on samples representing several stages of ripening. The solutions were prepared by washing the combined ether solutions, after 1 saponification, with water. None of the extracts were saponified until no further brown colour appeared. The extracts from 50 gm. of cut 6 and 85 gm. of cut 8 were saponified 3 times and there was an appreciable amount of brown colour after the third saponification. An arbitrary unit (brown unit) was used to calculate the relative amounts of the substance responsible for the brown colour. The results are tabulated in Table 4.

Table 4.—Relation of brown colour to stage of ripening of whole wheat meal

No. of cut	Date of cutting	Brown units	
		1 Saponification	3 Saponifications
1	July 20	7.2	—
3	27	10.5	—
4	31	8.8	—
5	Aug. 3	8.1	—
6	7	8.8	11.2
7	10	7.4	—
8	14	9.7	11.0
9	17	7.0	—

There was no correlation between the amount of the substance responsible for the brown colour and the stage of ripening. The amount of this substance was quite constant. Repeated saponifications yielded more of the brown colour but showed no variation in amount in different cuts.

Summary of Chemical Properties

Before treatment with alkali:

1. The substance responsible for the brown colour was not removed from whole wheat meal by extraction with 30% acetone or water.

2. Washing an ethyl ether solution with 25% methyl alcohol did not eliminate it.

3. It was not separated from the other pigments on a chromatogram.

4. It was soluble in anhydrous ethyl ether, petroleum ether and benzene.

After treatment with alkali:

1. The substance turned reddish-brown in colour and was soluble in ethyl ether, water and methyl alcohol, but not in carbon bisulphide.

2. It was readily forced from an aqueous phase to an ethyl ether phase by saturating the former with ammonium sulphate, phosphotungstic acid, or ammonium chloride.

3. Positive tests were obtained with the Folin and Denis reagent and with the Molisch test.

4. It had pronounced indicator properties, being yellow in acid solution and brown in alkaline solution.

5. Treatment of the brown solution with lead acetate gave a brown precipitate.

6. After the brown substance had been precipitated with sodium tungstate and sulphuric acid the clear filtrate reacted positively with the Folin and Denis reagent, the biuret test and xanthoproteic test.

The exact nature of the substance responsible for the brown colour is not known. Some of its properties indicated that it may have been a flavone pigment or pigments. Other properties suggested that it was protein. If the extracts can be freed from protein, the problem will be greatly simplified. Further investigation is being carried out.

SUMMARY

Samples of Regent wheat harvested at 10 different stages of ripening in 1939 have been analysed for chlorophyll, xanthophyll, and carotene. There was a marked reduction of all 3 pigments as the wheat matured. This was most marked in the case of chlorophyll and at the fully ripened stage the quantity of this pigment was less than 1 p.p.m. Xanthophyll still remained in the kernel to the extent of about 2.5 p.p.m. and carotene to 0.15 p.p.m.

Chlorophyll determinations were carried out on 5 samples of Regent wheat harvested at successive stages of ripening in 1940. As in 1939 there was a marked reduction of chlorophyll as the wheat matured.

A brown coloured substance which interfered with the chlorophyll determinations has been described, but its exact nature is not known. Studies on its chemical identity are reported.

A method for the determination of chlorophyll in whole wheat meal, which overcomes the difficulty due to the presence of the brown coloured substance, has been worked out.

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DEVELOPMENT OF THE LILY[1]

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Bulbs of the Bermuda lily, *Lilium longiflorum* var. *eximium* Nichols, subjected to storage before planting give a reduced number of blossoms. Increased storage further reduces the number of flowers. An investigation of the morphology of the developing lily was undertaken in order to determine whether in stored bulbs floral primordia were initiated and subsequently degenerated giving a final cluster of the reduced number of flowers.

MATERIALS AND METHODS

Bulbs of the lily packed in powdered coral were received from Bermuda on August 11, 1939. Two lots of these bulbs were set aside for the morphological examinations. Series I was planted in the greenhouse on August 12. A collection of 4 was made at that time and every 3 weeks thereafter until full flower bud formation. Series II was stored in coral in the dark for 12 weeks at 39° F. and then planted in the greenhouse. Collections were made every 3 weeks during storage and until 12 weeks after planting. Thenceforth collections were made at 18, 23, and 24 weeks.

Where the axis had not pushed through the bulb, the number of scales per bulb was counted. Notations were made on the number of leaves per plant. The length of the axis was measured. The terminal bud of the axis was removed, most of the leaves discarded, and the bud fixed in PFA_{15}. The collections were dehydrated through a Butyl alcohol series and embedded in paraffin. Transverse and longitudinal sections 15 microns in thickness were made with the rotary microtome. The stains used were, in general, hematoxylin, crystal violet, orange G and safranin. Photographs of the median longitudinal sections were made. This permitted comparative interpretation of the broadening and rounding of the terminal section of the axis.

RESULTS

The average number of scales on the stored bulbs was 71. No growth of the axis occurred during 12 weeks' storage. Growth of the axis occurred only after planting. A comparison of this axial growth of stored and unstored bulbs is given in Table 1.

Table 1 gives the average axial length at each examination. Considerable variation occurred in the individuals of each sample. Not enough bulbs were available to overcome these individual differences. This accounts for the reduction of the average axis length in those plants the bulbs of which were not stored and had been planted 21 weeks.

The unstored bulbs initiated new growth immediately after root establishment. This growth continued at a nearly steady rate until full bloom. After planting, the stored bulbs require a longer time for starting.

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TABLE 1.—GROWTH OF STORED AND UNSTORED BULBS

No Storage		Stored 12 weeks	
Planted	Growth	Planted	Growth
Weeks	Cm.	Weeks	Cm.
3	0.125	3	0.0
6	2.9	6	3.3
9	9.1	9	4.1
12	17.2	12	11.2
15	26.2	18	19.1
18	35.3	23	25.0
21	31.8	24	35.6

This is not due to lack of a root system for during the storage period there is root growth activity. Therefore, the delay in initiation of axial growth may be due, in part at least, to the decreased reserves in the bulbs.

Visible flower buds were formed on the distal point of the axis at 18 weeks in unstored bulbs and between 18 and 23 weeks in the stored bulbs. Five or 6 flower buds were formed on the plants from the unstored bulbs and not more than 3 on those from the stored material. The number of leaves on plants from Series I at flower bud formation averaged 130, and on Series II, 69.

The width of the terminal meristems obtained from the median longitudinal sections are given in Table 2.

TABLE 2.—WIDTH OF THE TERMINAL MERISTEM OF THE AXIS

Stored		Planted		Stored 12 weeks	
Weeks	Mm.	Weeks	Mm.	Planted, weeks	Mm.
0	0.37	0	0.37	0	0.37
3	.37	3	.39	3	.60
6	.43	6	.48	6	.67
9	.43	9	.47	9	.48
12	.46	12	.61	12	.67
		15	.80	18	.87
		18	.80		

It will be noted that though there is no perceptible lengthening of the axis during storage, the width of the terminal meristem increases slightly. The plane of the stem tip is either flat or very slightly convex. The convexity is no greater than 0.1 mm. A decided increase in the diameter of the terminal meristem occurs when the stored material is planted. In all stored material cell division occurred during storage, but mitoses are not as numerous as in any planted material. Since the diameter of the terminal meristem increases, and since a slight convexity of the growing point occurs, some growth occurs during storage. As mentioned previously, roots are formed during storage, but after the bulbs were held for 12 weeks at 39° F. there were fewer roots than after 3 weeks' planting without storage. At the 6 weeks' examination, the scales of the stored bulbs had lost their turgidity.

With 15 weeks' planting, no storage, buds were being initiated. The terminal meristem divided to form these flower buds. Such division was not visible macroscopically. The same was noted in plants, the bulbs of which were stored 12 weeks, planted 12 weeks. Therefore, flower bud initiation appears earlier in stored material compared with material planted immediately after arrival. This agrees with the repeated observations of A. W. S. Hunter of this Division that the first open flower from stored bulbs appears in a shorter time after planting than the first flower from unstored bulbs. Although the diameter of the terminal meristem of the stored material is greater during growth of the axis it does not follow that the stem also has a greater diameter. The terminal meristem covers only a small portion of the stem tip. A comparison of plants from unstored and stored bulbs shows the stem of the latter is almost always thicker than the former.

Globular bodies of an undetermined substance were present in the cells of the crown at the time the unstored bulbs were planted. A diminishing quantity of this substance was present in the crown of the bulbs stored 6 and 9 weeks. None appeared in the material stored 12 weeks nor in unstored bulbs planted 6 weeks and later. Unfortunately, no material was available for microchemical tests. It is evident that this substance is used up during storage or the early growth of the plant. It is possible that its disappearance in the stored bulbs may be correlated with reduction in height of plant and number of flowers per plant when compared with the unstored bulbs.

Pfeiffer (1) indicates that floral differentiation had begun 18 days after planting. The length of the axis at this time was 1.6 mm. No such floral differentiation occurred in the axis of similar bulbs at Ottawa. Division of the terminal meristem for the initiation of flower buds was not seen until the axis was 11.2 cm. long in the stored material. This was 84 days after planting and such plants had an additional 84 days' storage. In the unstored material floral primordia appeared in 105 days when the axis was 26.2 cm. long. However, the rounded apex of the axis was evident in the first examination; that is, 21 days after planting, but this meristem was giving rise to leaves alone. Therefore, it is believed that Pfeiffer's figures of buds with rounded terminal meristems should be interpreted as the vegetative and not as the reproductive stage.

Perianth parts did not develop until the terminal meristem divided to initiate the individual flowers; that is, the separated knots of meristem on the distal point of the axis are floral primordia. Differentiation of the floral parts does not occur until at least two groups of meristematic tissue are distinguishable. The smaller of these two knots of meristem is the first flower initial. The outer perianth parts of the first flower may be seen as projecting knobs of actively dividing cells even before the larger meristem has undergone division to form the remaining flower initials.

Phyllotaxy is evident in these meristems. The lowest bud is formed first and the uppermost or central bud terminates the spiral. This spiral arrangement of buds is not as clear in the material that had been stored since fewer flowers develop therefrom.

No formation of floral meristems followed by their degeneration and reabsorption was found in this investigation. The factors causing the reduction in the number of flowers associated with storage of the bulbs must lie farther back in the ontogeny of the plant.

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ROOT FIBRE PRODUCTION OF SOME PERENNIAL GRASSES[1]

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In Western Canada soil drifting has increased in extent and severity as the duration of settlement and cultivation has advanced. To-day it is one of the major problems confronting agriculture, but its importance is often not fully recognized. Its serious nature is most forcefully described by Whyte and Jacks (9) who clearly point out that the consequences of soil drifting are a loss in fertility, a reduction in water absorbing and water holding capacity, and an increased tendency to erode. The graveness of these effects is directly correlated with the extent of the erosion, and the original endowment of the soil is one of several factors which determine how rapidly the erosion will proceed and how soon its effects shall reach serious proportions. However, irrespective of soil type or location the fact that soil drifting causes a definite deterioration of the soil is indisputable.

Water erosion and increased droughtiness follow as a natural sequence of the effect of wind erosion in reducing the water absorbing and water holding capacity of the soil (9). The insidious water erosion has already been observed in many parts of Western Canada, and with continuation of present agricultural practices its ravages will undoubtedly increase. Drought, generally considered a cause of soil erosion, is also an effect of erosion. On eroded soils a greater proportion of the rainfall is lost to plant life through run-off and percolation. Whyte and Jacks (9) state that "In semi-arid countries, where every drop of rain is needed to maintain some life, this consequence of erosion is far more serious than the actual loss of soil." "The recent spells of drought years in North America, South Africa and Australia would not have been so devastating fifty years ago, as much more of the rain would have been held by the absorbent soil and utilized by the crops."

The basic cause of wind erosion is the small size of the soil particles or aggregates. Ellis (1) states that "Any soil in a powdery or in a 'single grain' condition will drift. Large aggregates, on the other hand, are not moved by wind." While on most soils drifting can be held fairly well in check by cultural practices, such procedures do not eliminate the above underlying cause of erosion, and at best provide only a temporary means of control. On the other hand the use of grasses has been shown to increase the size of soil aggregates (1, 4, 5, 6). However, it has been observed in a general way for some time that grass species differ in their ability to influence soil structure and control soil drifting.

Discussing the results of a survey of soil drifting areas in Manitoba, Harrison (2) stated that "Throughout the survey practically every farm which had grass in the rotation was overcoming the trouble to a great

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extent." "From a scrutiny of from 100 to 200 farms, we found that where brome was being grown, practically no drifting occurred, whereas in places where timothy and western rye grass were used the soil drifted."

In order to secure information on the relative root fibre production of slender wheat grass (*Agropyron pauciflorum*), crested wheat grass (*A. cristatum*), and brome grass (*Bromus inermis*), which are the three principal cultivated grasses of the drier areas of Western Canada, a project was commenced in 1935 at the Dominion Forage Crops Laboratory. Another objective of this study was to determine how long it would be necessary to leave land in grasses to secure a satisfactory restoration of root fibre. In this paper the results obtained to date from this project are presented.

MATERIALS AND METHODS

The regular variety test plots, in which all species were sown in drills spaced 6 inches apart, were utilized as the source of material. Two procedures were followed. In one method cubic foot samples were obtained in 4 three-inch layers by using a steel frame 1 foot by 1 foot by 6 inches, and securing in most cases 3, and in a few instances 6 samples from each plot. Most of the plots were one eighty-fourth of an acre in size. The locations sampled were selected to represent good stands. The steel frame was driven into the soil until level with the soil surface, and then the stubble was removed by cutting with a sharp knife just below the soil surface. The cubic foot sample was then removed in four layers (0–3″, 3–6″, 6–9″, and 9–12″) by means of a spade and trowel and placed in a cotton bag. Sampling in this manner was conducted each year from 1935 to 1940, utilizing in each year as many plots as were available of stands of different ages of each of the three grasses.

The other procedure consisted of digging a hole 4 feet square to a depth of 5 feet, on one side of which a column of soil a square foot in cross section was left protruding into the trench. After all surface growth was removed, the column was taken up in the following layers: 0–3″, 3–6″, 6–12″, 12–18″, 18–24″, 24-36″, 36–48″ and 48–60″. After placing the samples in cotton bags they were treated in the same manner as samples secured by the other method. Because of the labour involved, it was possible to use this method only in 1935 to secure data from one 4-year-old plot of each of the three cultivated grasses and one sample from each of ungrazed and lightly grazed prairie.

As soon as possible after obtaining the sample, washing was done in a low tank which was divided into two sections with a medium coarse screen in the partition between the two sections. The outlet at the end of the tank consisted of a fine screen. Before washing, the bags containing the samples were soaked for several hours so that in the washing the force of a coarse spray of water along with some rubbing was sufficient to break up all lumps of soil. The root fibre which was thus freed collected on the screens and was transferred to fine mesh screen baskets. Washing was continued until practically all soil was removed from the first compartment, and such soil as did remain was free of root fibre. After this process some soil particles still clung to the roots and some foreign organic matter was generally present. Further cleaning of the root fibre was thus necessary.

Cleaning was done by first spraying the fibre while still in the fine mesh screen baskets with a forceful spray of water to loosen the soil particles. The fibre was then transferred to a metal pan containing 3 or 4 inches of water, in which the soil settled to the bottom, and the floating foreign organic matter was scooped off the water surface. By careful manipulation the root fibre was returned to the wire baskets by pouring off the water, while at the same time the soil was retained. It was sometimes necessary to repeat this procedure.

The presence of rhizomes in the case of brome grass and of varying amounts of top growth and crowns in the uppermost layers (0–3″) of all samples presented a problem. The procedure adopted was to clip off and discard all crowns and underground stems. The data thus represent fibrous roots only.

After cleaning, the samples were placed in an oven drier and held at a temperature of 212° F. for 3 hours. As soon as the wire baskets were cooled sufficiently to permit handling, the absolute dry weight was determined to the nearest tenth of a gram. In the case of all cubic foot samples the root fibre yields were converted to pounds per acre foot.

In a study of root diameter and tensile strength, only living roots were used. The roots were excised just prior to treatment and were kept in water. The diameter was measured by means of a microscope containing an ocular micrometer. The weight in grams required to break the root was designated as the tensile strength.

EXPERIMENTAL RESULTS

The root fibre yields, as determined from the cubic foot samples and expressed as pounds per acre (dry weight) to a depth of one foot, are summarized in Table 1.

Table 1.—Comparison of root fibre production of three grasses by age of stand

Age of stand (yrs.)	Total No. of samples averaged			Average root fibre yield in lb. per ac. ft.		
	Crested wheat Fairway	Brome Comm.	Slender wheat	Crested wheat Fairway	Brome Comm.	Slender wheat
				lb.	lb.	lb.
1	18	21	27	2665	2898	1778
2	18	21	24	4049	3963	2113
3	21	18	21	5244	4657	2678
4	21	15	21	5968	5255	2471
5	12	6	15	6229	5768	2901
6	3	—	—	7432	—	—
7	6	—	—	8196	—	—
8	3	—	—	8055	—	—
9	—	—	—	—	—	—
10	—	3	—	—	8803	—

Root fibre yield of lightly grazed native prairie (Av. of 7 samples) = 12,850 lb. per acre foot.

Considering crested wheat grass alone, it is seen that this grass adds each year to the weight of root fibre present in the top foot of soil. Five-year-old stands have built up about one-half, and 7- and 8-year-old stands

about two-thirds the quantity of root fibre present in lightly grazed native prairie. While it may appear that the rate of increase is becoming less rapid as the stand ages, this is probably due to chance variations rather than a real difference.

Brome grass develops in a manner similar to that of crested wheat grass in that it continues to build up root fibre year after year at a fairly rapid rate. The average root fibre yield of brome grass stands of 2 to 5 years of age is slightly less than that of crested wheat grass stands of the same age. Approximately one-half of the sampled plots of these two crops were closely adjacent, and the data for these plots lend themselves to analyses by Student's Pairing Method. The P value for the 12 paired comparisons was found to be between .2 and .1, indicating that the difference between the crops was not significant. Pavlychenko (5), on the other hand, based on the detailed analyses of one 2-year and one 3-year-old plant of brome grass, concluded that the root system showed signs of deterioration from the second to the third year, whereas in a similar analysis of crested wheat grass he found that the root fibre increased from the second to the third year.

Compared to the other two grasses, the root fibre yield of 1-year-old stands of slender wheat grass is much lower and in subsequent years does not build up nearly so rapidly. Five-year-old stands produced on the average only about as much root fibre as 1-year-old stands of brome grass and crested wheat grass. Thus slender wheat grass clearly produces significantly less root fibre than the other two grasses. From general observations it appears that the roots of this species are short lived, probably a high proportion of them dying each year. Whether death is due to infection by rootrot organisms or a natural death followed by infection is unknown, but rootrot lesions were commonly observed. The low root fibre yield of slender wheat grass at least partially explains the fact that sod of this grass breaks down upon plowing, almost like cereal stubble.

An indication that strains as well as crops may differ in root fibre yield was obtained by comparing the common and Fairway types of crested wheat grass, the data for which are given in Table 2.

TABLE 2.—COMPARATIVE ROOT FIBRE YIELD OF PLOTS OF FAIRWAY AND COMMON CRESTED WHEAT GRASS LOCATED CLOSE TO ONE ANOTHER

Age of stand (yrs.)	No. of plots averaged	Average root fibre yield in lb. per acre foot	
		Common type	Fairway type
1	3	2661	2908
2	2	4002	3994
3	2	4692	5659
4	2	5219	6182
5	1	6615	6242
6	1	5928	7432
7	1	6562	8023
8	1	7140	8055

Table 2 shows that in older stands the root fibre yield of the Fairway type is higher than that of the common type. Analysis of the data by Student's Pairing Method gave a *P* value of less than .01, indicating that the differences are significant. Stevenson and White (7) have earlier reported a similar difference.

Although differences existed in the total yield of root fibre of the three grasses, its distribution in the top foot of soil was practically identical for all grasses, as is shown by Table 3.

In the case of each species about 50% of the total roots of the top foot are contained within the top 3 inches, around 25% in the 3–6″ depth, 15% in the 6–9″ depth and 10% in the 9–12″ depth. Thus brome grass and crested wheat grass not only produce practically the same weight of roots per unit area but have practically the same weight in different layers. There is no indication to be found in Table 3 that the distribution of root fibre in the soil is affected by age of stand. The increase in total weight with ageing of stand takes place uniformly in the top foot of soil.

Table 3.—Distribution of root fibre by layers in top foot of soil

Age of stand (yrs.)	Crested wheat grass Percentage of roots in layer				Brome grass Percentage of roots in layer				Slender wheat grass Percentage of roots in layer			
	0–3″	3–6″	6–9″	9–12″	0–3″	3–6″	6–9″	9–12″	0–3″	3–6″	6–9″	9–12″
	%	%	%	%	%	%	%	%	%	%	%	%
1	50.06	27.94	13.16	8.84	52.38	25.60	13.05	8.97	53.63	26.56	11.46	8.36
2	51.15	25.58	13.31	9.96	52.39	24.41	14.51	8.69	50.71	28.63	11.87	8.80
3	46.26	25.18	17.51	11.04	46.66	26.00	17.39	9.96	45.54	25.55	18.78	10.14
4	52.21	24.88	14.31	8.60	48.09	27.30	14.68	9.94	48.83	26.02	14.11	11.03
5	49.73	25.63	13.82	10.82	48.83	29.64	13.04	8.49	56.97	24.37	11.24	7.42
6	45.70	21.17	19.17	13.96	—	—	—	—	—	—	—	—
Av.	49.83	25.37	14.79	10.01	49.79	26.17	14.73	9.31	50.35	26.55	13.75	9.36

The distribution of roots in the soil to a depth of 5 feet is given in Table 4. The data in this table were obtained from single samples, $1' \times 1' \times 5'$ from each of the cultivated grasses and from grazed and ungrazed prairie.

Table 4.—Distribution of root fibre by layers in the top 5 feet of soil

Crop	Age of stand	Total wt. of roots in column 1′× 1′× 5′	Percentage of total roots in layer				
			0–12″	12–24″	24–36″	36–48″	48–60″
	yr.	gm.	%	%	%	%	%
Grazed prairie	—	209.10	76.95	18.36	3.30	0.96	0.43
Ungrazed prairie	—	154.65	91.37	5.17	2.07	0.77	0.61
Crested wheat grass	4	89.65	88.18	6.02	2.34	1.90	1.56
Brome grass	10	122.05	87.75	6.72	2.74	1.68	1.11
Slender wheat grass	4	53.95	89.16	6.76	2.04	1.30	0.74

In the case of the cultivated grasses, the results in Table 4 show that approximately 88% of the total weight of roots is contained in the top foot of soil, about 7% in the second foot, and around 1.5% or less in the 4 to 5 foot layer. There is apparently little difference between species in this respect, although there is some indication that slender wheat grass has a smaller proportion of its roots in the lower layers. Pavlychenko (5) concluded that these three grasses differed in the distribution of their root fibre, particularly in the 0–6″ layer, less so in the 6–12″ layer, and very little in the 12–60″ layer.

It is seen from Table 4 that the lightly grazed and ungrazed prairie differed quite markedly in the total root fibre yield and its distribution. Since these data were obtained from single samples in each case, it may be that these differences are due to chance fluctuations. Comparing the distribution of the roots in the prairie sod with those of crested wheat grass and brome grass sod, it is seen that the prairie sod had a lower percentage of roots in the 36–60″ layer than did these cultivated grasses. This does not necessarily indicate that the cultivated grasses have a deeper root penetration than the native grasses. The soil moisture under native prairie may have been exhausted to a greater extent than under the rather young stands of cultivated grasses, and this factor would undoubtedly influence root penetration.

It was observed by Kirk *et al.* (3) that the fibrous roots of crested wheat grass were much stronger than those of slender wheat grass. During the course of the present investigation such differences were also observed, crested wheat grass roots not only being more difficult to break but also to cut than the roots of the other two grasses. To obtain a quantitative measure of these observed variations the tensile strength of the roots of the three grasses was determined, the results of which are summarized in Table 5.

TABLE 5.—AVERAGE DIAMETER AND TENSILE STRENGTH OF LIVING ROOTS OF THREE GRASSES

Grass	No. of roots used	Average diameter of roots in units*	Av. weight in grams required to break roots	
			Absolute	Per unit diameter
			gm.	gm.
Crested wheat	35	18.60 ± 0.32	569.5 ± 25.67	31.11 ± 0.99
Brome	35	15.94 ± 0.36	439.6 ± 23.78	27.30 ± 1.04
Slender wheat	36	12.69 ± 0.25	271.0 ± 16.56	20.94 ± 0.97

* Unit equals about 0.016 mm.

Whether expressed on an absolute basis or on the unit diameter basis, these data show that crested wheat grass roots were considerably stronger than those of brome grass, while slender wheat grass roots are much weaker than those of either of the other grasses. The odds that these differences are significant are in excess of 1000 to 1 for all comparisons except that of brome and crested wheat grass on the unit diameter basis where the odds

are 100 to 1. Although differing in average diameter, this factor had no measurable effect on strength of root. Pavlychenko (5) found similar differences in tensile strength of the roots of these three crops, and that differences in diameter had little or no effect on the tensile strength of roots of unrelated species, although within the species the strength was fairly proportional to the diameter of various root structures.

Assuming that the specific gravity of roots is constant between species, certain inferences respecting root length can be drawn from a knowledge of the average weight of roots produced and the average diameter of those roots. It is shown in Table 5 that highly significant differences exist in the average root diameter of the three species under consideration, crested wheat grass having the largest and slender wheat grass the smallest roots. It has also been shown that brome grass and crested wheat grass of the same age did not differ significantly in the weight of root fibre produced. On the basis of these facts and assuming that the specific gravity of the roots of these two species is the same, it may be concluded that the length of root fibre produced by brome grass in the top foot of soil is at least equal to if not greater than that of crested wheat grass, irrespective of age of stand. On a similar basis it may be inferred that the length of root fibre produced by slender wheat grass is less than that of the other two grasses. However, since the diameter of slender wheat grass roots is less than that of the other two grasses, it seems likely that the differences in length of root fibre of this species, as compared to the other two grasses, is not as great as indicated by the weight of roots produced. These conclusions are contrary to certain results presented by Pavlychenko (4, 5).

In a study of root fibre from blocks of sod, Pavlychenko found that the weight of roots produced by crested wheat grass was very similar to that of brome grass, but that the length of the root fibre of crested wheat grass was about 250% greater than that of brome grass. On the basis of these data, it may be inferred that the above assumption of equal specific gravity for the roots of these two grasses is not well founded. This factor needs further investigation. In keeping with the above conclusion that the length of root fibre of slender wheat grass more closely approximates that of the other two grasses, Pavlychenko has shown that the weight of root fibre produced by slender wheat grass is very much less than that of the other two grasses, but that the length of roots is similar to that of brome grass.

DISCUSSION

In the results presented above it has been shown that the weight of root fibre per acre foot produced by slender wheat grass is definitely less than that of brome grass and crested wheat grass. From the standpoint of adding root fibre to the soil this former species is thus inferior. In addition, the plants of this species are short lived, and observations indicate that the roots decompose readily. Brome and crested wheat, on the other hand, produced comparatively large quantities of root fibre, and there is no indication that this root fibre is short lived. Analyzing a 3-year-old

plant of crested wheat, Pavlychenko (5) found over two-thirds of the crown roots were old and still functioning. Stoddart (8) in preliminary work showed that both nodal and seminal roots of prairie grasses may live in excess of two years, even under adverse conditions.

While quantity of root fibre is undoubtedly one factor which contributes to changes in soil structure, there is evidence to indicate that it is not the only factor. Slender wheat grass sod is known to break down almost as readily as cereal stubble. This is clearly shown in Figure 1. Crested wheat and brome, on the other hand, produce a much superior sod, as is shown in the case of crested wheat grass in Figure 1. This difference between slender wheat grass and the other two grasses may actually be attributed to differences in quantity of root fibre. However, brome grass and crested wheat grass, while not differing in the weight of root fibre, produced in the top foot of soil, have been observed to have different effects upon soil structure.

FIGURE 1. Newly broken sod of crested wheat grass (right) and slender wheat grass (left). Sown 1935, broken 1939.

In excavating the samples for washing, those from crested wheat grass were usually much harder to dig than those of brome grass. It was often necessary to use a pick in the case of crested wheat grass samples to loosen and break up the soil, while this was seldom necessary for the brome grass

samples. In plowing a series of plots in which both grasses were represented, it obviously took more power to plow the crested wheat grass, and its sod formed a decidedly better ribbon than did that of brome grass. The brome grass sod had more of a tendency to break. From these observations it is obvious that it is the quality or nature of the root fibre, as well as the quantity, that is of importance in altering soil structure. Pavlychenko (5) points out that wheat ranks high as regards the quantity of fibre produced, but is far inferior to the grasses in quality of fibre. By determining the size of soil aggregates in sods of various grasses and from continuous wheat and a wheat oat rotation, Pavlychenko (5) found that in the 0 to 10 centimeter level the aggregates 4 to .8 mm. in size represented 0.61% of the total in continuous wheat, 15.23% of the total in crested wheat grass sod, 0.16% of the total in slender wheat grass sod, and 2.09% of the total in brome grass sod. The virgin soils were found to be composed of from 14 to 20% of aggregates 4 to .8 mm. in size. Pavlychenko's results clearly indicate that crested wheat grass is vastly superior to either of the other two grasses in building up large soil aggregates which will be of value in resisting soil erosion. However, much further investigation on this matter is needed, for while there are data to indicate that crested wheat grass is superior to brome grass in improving soil structure, the general observation has been that the sod of brome grass provides considerable protection against erosion, and its use for this purpose can hardly be discouraged in the light of the rather limited information so far available. But all investigations and observations conclusively agree that slender wheat grass is of little or no value for this purpose.

Pavlychenko (5) has concluded that a close relationship exists between the amount and strength of roots and their ability to restore soil structure. On the basis of data presented in this paper, there is little evidence of this relationship. Crested wheat grass was not found to differ from brome grass in the amount (weight) of roots produced. While the roots of crested wheat grass were found to be stronger than those of brome grass, it seems improbable that the magnitude of this difference is sufficiently great to account for the marked difference in effect of these two grasses on soil structure, as reported by Pavlychenko (5). It would appear that crested wheat grass exerts its superior beneficial effect on soil structure in some as yet unexplainable manner.

A definite statement on how long land should be left sown down to crested wheat grass or brome grass to effect a reasonably good restoration of root fibre is difficult to make. It has been shown in Table 1 that both of these grasses continued to build up root fibre as far as the investigation proceeded. Then, too, the root fibre restoration would undoubtedly be materially influenced by soil and climatic factors. However, on the clay loam soil at Saskatoon 5- or 6-year-old stands have restored approximately half the fibre contained in native prairie sod, and Pavlychenko (5) has show that the proportion of larger soil aggregates from 5-year-old stands of crested wheat grass is approximately equal to that of virgin prairie. In view of these facts, it appears that for clay loam soils at Saskatoon 5-year-old stands of crested wheat grass will provide a satisfactory restoration of soil structure. The sod of brome grass from 5-year-old stands is inferior to that of crested wheat grass, and it is impossible to state whether older

stands of brome grass will ever equal that of 5-year-old stands of crested wheat grass. The optimum length of ley will, however, vary with environmental conditions since climatic factors and the soil texture and condition at the time of seeding will have a pronounced influence on the restoration of soil structure. In this connection, Ellis (1) points out that it is probably never possible to produce a satisfactory structure to sandy soils, and consequently soils of that type should be left in grass.

From the evidence obtained in the present investigation and from the excellent data on soil structure changes presented by Pavlychenko (5), it is clear that grasses can and should be used extensively in Western Canada as a means of building up the soil to prevent soil erosion. The practice of including grasses in rotations would not necessarily involve a drastic change from the present wheat farming methods. The inclusion of grasses in the rotation with the object of improving the soil may well be looked upon as a means of enabling the farmer to continue wheat growing through the control of erosion and maintenance of fertility, as well as by aiding in the control of weeds, and insect and disease pests.

SUMMARY

1. The results are presented of a study of the quantity and distribution of root fibre produced by stands of various ages of crested wheat grass, brome grass, and slender wheat grass as compared to native prairie.

2. Crested wheat grass adds each year to the root fibre present in the top foot of soil, and 5-year-old stands have built up over 3 tons of root fibre (dry weight) per acre foot, which is about one-half the quantity of root fibre present in native prairie.

3. Root fibre yields of brome grass are very similar to those of crested wheat grass.

4. Slender wheat grass produces much less root fibre than the other two grasses.

5. For the three cultivated grasses the proportion of root fibre present in the 0–3″, 3–6″, 6–9″, and 9–12″ layers was found to be approximately 50, 25, 15 and 10% respectively, and was not influenced by species or age of stand.

6. The root fibre distribution of the three cultivated grasses was found to be approximately 88% in the top foot, and 7% in the second foot, with a decreasing proportion as depth increased to 5 feet.

7. A significant difference was shown in the root diameter and tensile strength of the living roots of the three grasses, crested wheat grass having the largest and strongest roots and slender wheat the smallest and weakest.

8. Crested wheat grass is considered to be superior to brome grass in increasing the size of soil aggregates. Slender wheat grass is considered of little or no value in improving soil structure.

ACKNOWLEDGMENTS

To C. A. Rowles, W. T. Burns, R. G. Savage, F. E. Payne and E. Buglass who have at various times conducted the sampling, washing, cleaning and weighing, the authors are deeply indebted, and their assistance is gratefully acknowledged.

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A NECROTIC "FERN-LEAF" MOSAIC OF RASPBERRY[1]

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In May, 1935, a single 3-year-old Cuthbert stool in one of the laboratory plantations showed peculiar symptoms suggestive of virus infection but unlike those of the known virus diseases of the raspberry. At the time an experiment in the transmission of viruses by the panel method of patch grafting was under way, and "transfers" from this stool were included to determine the transmissibility of the symptom complex. On the basis of experimental evidence submitted in this paper the condition has been designated necrotic "fern-leaf" mosaic.

OCCURRENCE

Up to the present time necrotic "fern-leaf" mosaic is not known to occur in commercial plantings in Ontario and is, therefore, not of economic importance. Diseases with somewhat similar symptoms have been reported in Pennsylvania by Zundel (8) and in British Columbia by Jones (4). Zundel reported a "fern-leaf" condition on Cumberland, in which leaves were small, narrow and with accentuated serrations, accompanied by a marked dwarfing of cane growth. He attributed the occurrence of the diseased Cumberland plant to chance infection with the virus of Cucurbit mosaic. Jones has observed in raspberries a condition characterized by retarded foliation and the appearance of necrotic spots on the leaves which he considers to be due to virus infection. These symptoms are also found on plants infected with necrotic "fern-leaf" mosaic, but whether these diseases are otherwise similar is not known.

SYMPTOMS

Symptoms of this disease have four characteristics, which, although they may show considerable variation, have been found sufficiently definite and distinctive to warrant considering this a new disease.

1. *Mottling* (Figure 1). Foliage mottling ranges from distinct yellow spotting and ring-spotting, to extensive, coarse, well-defined pale green to yellow blotches. Sometimes only part of a leaf or leaflet may show the markings, and spotting may be limited or profuse. Where the mottling is extensive it resembles closely the severe form of green mottle mosaic. Mottling may be observed most readily in June on the younger leaves of the current season's canes, but becomes partially or completely masked during the warmer weather of mid-summer and though it appears again on late season growth, it is more indefinite. The mottling on the foliage of fruiting canes lacks the definite character found on younger canes.

2. *Necrosis.* Necrotic spotting may be found more or less extensively on the older basal leaves of newly infected canes and is generally associated with the yellow spotting. Sometimes the small, necrotic spots run together

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FIGURE 1. Foliage mottling of necrotic "fern-leaf" mosaic on Viking.

and involve larger areas of the leaf tissue in which case the leaf shows a downward and inward rolling. In the red varieties this necrosis has been confined to the leaf tissues and appears principally in the early stages of the infection.

3. *Stunting.* Both cane and leaf are rather markedly stunted. The leaves are small and narrow with accentuated serrations (fern-leaf) (Figure 2), especially at the tip of young canes. This is particularly noticeable on young sucker plants, so much so that it is frequently difficult to identify the variety by its leaf characteristics.

4. *Retarded foliation.* The bud development of fruiting canes shows great irregularity, some developing normally, some exhibiting a marked delay in foliation, while others fail to grow. Buds break slowly, the leaves remain clustered and are slow to expand and in this respect are somewhat similar to canes injured by winter temperatures. Growth of fruiting laterals is slow and stunted attaining only one-third the growth of normal canes. Cooley (3) found a similar delayed foliation in a high percentage of black raspberry plants infected with green mottle mosaic.

Diseased stools continue to grow poorly for a number of years but present a thin appearance due to a lack of stooling, to small leaves, and to stunted cane growth.

EXPERIMENTAL WORK

The technique of transmission of this disease was the same as that used in the study of yellow blotch curl (2), namely the panel type of patch grafting.

FIGURE 2. Three-year-old Cuthbert stools, (left) infected with necrotic "fern-leaf" mosaic, and (right) healthy stool. Note size and fern-leaf character of foliage and stunted lateral growth of the infected cane.

Two grafts were made from the original affected Cuthbert stool to healthy clonal Viking plants on June 10, 1935. On July 27 (47 days later), the older leaves of a low basal lateral of one of these plants showed a light yellow mottling and a distinct and rather extensive necrotic spotting. Similar symptoms appeared later on a second lateral, while the main cane, two other laterals and the second grafted plant remained normal. Early the following spring both stools were delayed in foliation and with the appearance of a prominent blotchy mottling and a necrotic spotting of older leaves which showed a sharp downward rolling, it became evident that the stools were systemically infected with a virus transmitted by grafting from the Cuthbert stool. Both these stools remained stunted in 1936 and 1937, attaining a growth of only 2 feet. The behaviour of these stools, particularly the retarded foliation, the small, narrow, fern-like leaves and the drastic stunting of growth, was similar to that noted on the original diseased stool and from them material was obtained for many of the later grafts, carried on over a period of four years, which resulted in repeated successful transmissions.

A total of 122 grafts were made to young healthy canes of different varieties of both red and black raspberries and resulted in 90.1% transmission of the disease. The Cuthbert and Viking varieties proved highly susceptible, showing marked stunting with foliage symptoms of spotting, ring-spotting, blotchy and pattern mottling and necrosis. Spot necrosis was more prevalent on the Viking and was found principally associated with the yellow spotting, although it also occurred independently. Stunted, narrowed and fern-leaf foliage was more conspicuous on the Cuthbert. Both varieties showed a marked retardation of foliation, the young leaves

remaining bunched or rosetted for a prolonged period. Other varieties were grafted and found susceptible but varied in their reaction, the Latham, Brighton, Ulster, and Taylor responding with well marked symptoms and moderate reaction, while Marcy, Chief, Newburgh and Herbert varieties showed both mild symptoms and reaction.

Necrotic "fern-leaf" mosaic was successfully transmitted to 10 black raspberry stools of varieties Plum Farmer, Dundee and Naples. These grafts were made on June 7, 1937, but only one plant developed symptoms during that season. In May, 1938, however, all the plants showed a marked delay in bud development and foliation. The leaves later showed extensive mottling, with a rugose or crinkly effect, definitely more uniform and less contrasty than on red varieties. Considerable necrosis developed, especially on Naples variety, not only on the leaves but also to some extent on the tips of the fruiting spurs. The growth of the plants was very seriously stunted, vigour declined, new shoots failed to develop and the majority of plants died before fruiting. Failure followed attempts to transmit the disease from the infected black raspberries to Viking red raspberries.

Necrotic "fern-leaf" mosaic has proved to be very readily transmissible by patch grafting. The time necessary for symptoms, which invariably appeared on laterals or early sucker growth, varied from 15 to 75 days with an average of 27 days, a shorter period than was necessary for other viruses transmitted by this method. In some instances symptom expression was delayed until the following spring when the infection was systemic. Attempts to transmit this disease by means of the insect vectors *Amphorophora rubi* and *Aphis rubiphila*, known to transmit other viruses of the raspberry, were unsuccessful. Unsuccessful attempts to transmit the virus to other rosaceous hosts by grafting and to tobacco by juice transfers were also made.

THE EFFECT OF THE DISEASE

The principal effect of the disease is a marked reduction in the stooling ability of the plant and a dwarfing of fruiting laterals and new cane growth. Infected stools remain thin, stunted and continue to grow poorly for several years.

TABLE I.—CANE PRODUCTION AND GROWTH MEASUREMENTS OF THE FOUR GREATEST CANES PER STOOL ONE YEAR AFTER GRAFTING, NOVEMBER, 1939

Variety	Disease	Number stools	Number canes	Average cane per stool	Average height of canes in ins.	Average diameter of canes in $\frac{1}{16}''$
Viking	Normal	21	316	15.0	75.7	8.9
Viking	Fern-leaf M.	20	101	5.0*	49.2	6.6
Viking	Green M. M.	10	79	7.9†	61.5	7.9
Cuthbert	Normal	17	196	11.5	68.2	9.5
Cuthbert	Fern-leaf M.	5	36	7.2	43.4	5.6
Cuthbert	Green M.M.	3	33	11.0	53.1	6.7

*One stool died and 3 stools with less than 4 canes.
†One stool less than 4 canes.
M.=mosaic; MM.=mottle mosaic.

The berries from diseased canes were small, dry, seedy and tended to be more acid in flavour than normal berries.

NECROTIC "FERN-LEAF" MOSAIC, A DISTINCT DISEASE

It is known that when viruses of red raspberry mosaic and yellow mosaic are mixed together in one plant, the symptoms of both viruses develop (7). Workers with tobacco, potato and tomato viruses have demonstrated an acquired immunity in plants infected with strains of a virus in which such plants fail to produce symptoms when inoculated with closely related strains (1, 5, 6). The question, therefore, as to whether necrotic "fern-leaf" mosaic was the result of symptom modification induced by a virus complex or a particularly virulent strain of green mottle mosaic was investigated.

Four healthy plants each of Cuthbert and Viking were grafted with both necrotic "fern-leaf mosaic" and "green mottle mosaic", and symptoms of both diseases developed in all 8 instances. The regular and more uniform mottling of "green mottle mosaic" predominated and masked the irregular spot type of mottling of the other, especially in late season growth. Necrotic "fern-leaf" mosaic, however, could definitely be identified by the occurrence of leaf necrosis and delayed foliation the following spring. These plants showed a more serious stunting and a more severe reaction than plants infected with either virus alone. The first appearance of necrosis was on the Vikings and was evident 10 to 20 days before mosaic mottling. On the other hand, on Cuthbert, mosaic mottling appeared before necrosis which was less extensive than on Viking. Both varieties the following year showed retarded foliation and severe stunting of leaf and cane more marked on Viking than on Cuthbert.

Necrotic "fern-leaf" mosaic was also transmitted to Viking and Cuthbert plants known to be systemically infected with green mottle mosaic. Extensive necrosis of the older leaves appeared 41 days later on the Vikings, while on Cuthbert this symptom, while less extensive, was noted as early as 22 days after grafting. Although one Cuthbert plant failed to develop necrosis, the fact that foliation was retarded suggested positive transmission. The following year 25 grafts from these doubly infected plants were made to Viking and Cuthbert plants. Twenty-four instances of necrotic "fern-leaf" mosaic resulted from these grafts and 16 definite transmissions of green mottle mosaic, 2 doubtful cases, and 7 without symptoms of this disease. The presence of necrotic "fern-leaf" mosaic was in all cases readily determined either by the occurrence of necrosis, which was again more abundant on Viking, or delayed foliation and the "fern-leaf" character of the new leaves. It proved more difficult, however, to separate out true mosaic symptoms possibly because of seasonal conditions and a lack of vigorous young growth on which such symptoms can be detected most readily. However, plants showing diffuse mottling, greater pallor of leaf colour and stunting of cane in addition to necrosis were regarded as doubly infected. These experiments suggest that these diseases are distinct and caused by unrelated viruses. The earlier and more profuse development of necrosis on the Viking indicates greater susceptibility of this variety to necrotic "fern-leaf" mosaic. Apparently the virus spreads throughout the plant more rapidly than green mosaic. It was also true with some of the Cuthbert plants, although the tendency was not as marked. This may possibly be associated with vigour of growth, the Viking being naturally a more vigorous grower.

CONCLUSION

The results of these experiments afford conclusive evidence that a virus disease with well defined symptoms has been repeatedly transmitted by patch grafting. On susceptible varieties the outstanding characters which serve to distinguish it from other raspberry virus diseases are the development of spot necrosis of the older leaves, retarded bud development and foliation, ring-spot and blotch mottle, and an unusual stunting of the young leaf growth which results in a "fern-leaf" appearance.

Because the disease has not been found elsewhere in Ontario and no natural spread has been observed either from the original diseased stool or during the course of the investigation, it is considered likely that it represents a chance infection contracted from a nearby unusual source. It is presented, therefore, as a matter of interest as well as record of the occurrence of unusual symptoms of virus infection of the red raspberry.

SUMMARY

1. A virus disease of the raspberry causing a necrotic spotting of the leaf, marked retardation of foliation, and an irregular blotch and spot type of mottle is described.

2. The disease has been readily transmitted to *Rubus* species by the panel type of patch grafting but has not been transferred by insect vectors.

3. The Cuthbert and Viking varieties proved most susceptible.

4. Plants infected with a combination of necrotic "fern-leaf" mosaic and green mottle mosaic exhibited symptoms of both diseases.

5. The disease as yet is not of economic importance and is thought to represent a chance infection from an unusual source.

ACKNOWLEDGMENT

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BOOK REVIEW

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NEW PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

IMPERIAL BUREAU OF SOIL SCIENCE TECHNICAL COMMUNICATION No. 40
"The Rothamsted Field Experiments on the Growth of Wheat"

by

Sir E. J. Russell and D. J. Watson

1940. Pages 163. PRICE: 7s. 6d.

A full and up-to-date account of the famous 98-year-old continuous-wheat experiment on Broadbalk field, Rothamsted, and of other long-period experiments on the growth of wheat in rotation, on the results of which present-day practices in the use of artificial fertilizers are largely based. The Communication is in eight chapters: I. The Broadbalk experiments; II. Seasonal variation of yield and its causes; III. Deterioration of yield of wheat grown continuously; IV. Comparison of the results with those obtained under other conditions; V. Wheat grown in rotation; VI. Practical problems of wheat growing; VII. The development of the wheat plant, and its relation to yield; VIII. Effect of season and manuring on the growth and composition of the wheat plant.

Bibliography of Soil Science, Fertilizers and General Agronomy, 1937-1940

Summer, 1941. PRICE: 25s.

The third volume of this series, to be published during the summer. 1941. It will contain some 7,000 classified references with full indexes, The book will also serve as a cumulative subject index to Vols. I to III of the Soil Bureau's abstract journal, *Soils and Fertilizers.*

IMPERIAL BUREAU OF ANIMAL NUTRITION
TECHNICAL COMMUNICATION No. 14

The Efficiency of Farm Animals in the Conversion of Feeding-Stuffs to Food for Man

by

I. Leitch and W. Godden

February, 1941. PRICE: 3s. 6d.

The efficiencies of cattle, sheep, pigs and poultry in the production of milk, meat and eggs are compared. Efficiency is calculated in terms of dry matter, digestible feed constituents and edible human food. The calculations are based on accepted feeding standards and on experimental and practical rations. The effects on efficiency of level of production, of the nature of the ration, e.g. winter and summer feed, and especially the level of protein intake, and of the overhead charges for the maintenance of the individual and of the breeding stock are analyzed. Relevant data from the literature are reviewed. Where comparable data exist, chiefly for energetic efficiency, the present estimates are in good agreement. They extend the information by including calculations of the efficiency of protein and fat production and by comparing the estimated total feed supply of the country for one year with the total yield of human food.

IMPERIAL BUREAU OF PLANT BREEDING AND GENETICS

Potato Collection Expeditions in Mexico and South America

by

J. G. Hawkes

April, 1941. PRICE: 3s.

During the first eight weeks of 1939 an expedition was sent to South America by the Imperial Agricultural Bureaux to make collections of indigenous wild and cultivated potatoes. A growing need had been felt by potato breeders both in Britain and the Dominions and Colonies for fresh breeding material in order to introduce disease immunity into our domestic stocks and to produce varieties better adapted to the various ecological conditions within the Empire where the potato was grown or into which it was hoped to introduce it. The present bulletin embodies a full description of this expedition and of a subsidiary expedition to Mexico undertaken with the same objects. A full account is given of the itinerary, of the conditions in the countries visited, of the methods of native potato cultivation, and of the utilization of the potatoes by the natives in the preparation of chuño and other productions, the different countries being dealt with separately, since conditions vary from one to the other.

The author ends with a brief outline of the work now in progress on the further study and utilization of the large collection of Central and South American potatoes, under the heads of systematics and cytology, disease resistance, frost resistance, drought resistance, photoperiodicity, yield, short dormancy and culinary quality.

The bulletin is well documented, containing 11 tables, 2 maps, 1 half-tone plate and a bibliography.

New and Promising Varieties recently described in the Literature
(Third List)

April, 1941. PRICE: 1s.

In examining the literature on plant breeding it frequently happens that reference is seen to the production of some new variety of crop plant possessed of special qualities that make it of possible interest not only to growers in the country concerned, but also to plant breeders in other countries who may be occupied with similar problems. It has been suggested that in such cases the Bureau would be performing a useful service in drawing the attention of readers to these varieties, so that they may, if desired, take steps to procure material or more details of the new variety direct from the producer.

List of varieties recently produced have been prepared in 1939 and 1940 with this object in view; indications are given of the main qualities distinguishing the new variety, of its origin and the source from which a detailed description of it can be obtained (where mention has been made of the variety in "Plant Breeding Abstracts", the reference to the volume and abstract is given) and of the name of the producer when known. These lists were welcomed by a large number of readers, many of whom wrote and asked that they might be continued.

IMPERIAL BUREAU OF PASTURES AND FORAGE CROPS
BULLETIN No. 30

The Grasslands of the Argentine and Patagonia

by

W. Davies.

November, 1940. Pages: 48. PRICE: 2s. 9d.

A report of a tour of South American grasslands made during March and April, 1938, describing the eight grassland zones, and giving detailed notes on visits to individual Stations in Patagonia and Argentine proper.

IMPERIAL BUREAU OF HORTICULTURE AND PLANTATION CROPS
OCCASIONAL PAPER No. 6

Haricot Beans

by

G. St. Clair Feilden

February, 1941. Pages: 20. PRICE: 1s.

This bulletin has been compiled at the request of the Ministry of Agriculture, London, to help those intending to grow haricot beans for private or commercial purposes in Great Britain.

In the past, unlimited cheap supplies of beans from Japan, Hungary and North America have discouraged the farmer from putting his money into a crop which in England is only likely to achieve full success in years of dry summer.

But in wartime with overseas supplies greatly reduced, it has seemed reasonable to examine the possibility of providing a valuable addition to

the national menu by growing those varieties, which, by their yields of 1 ton or more per acre in 1940, have shown a certain adaptability to English conditions.

The bulletin contains a brief account of work with haricots in U.S.A. and Canada and of the results of experiments in this country at Wisley, Cambridge and elsewhere. Cultivation methods are outlined and varietal characteristics discussed.

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